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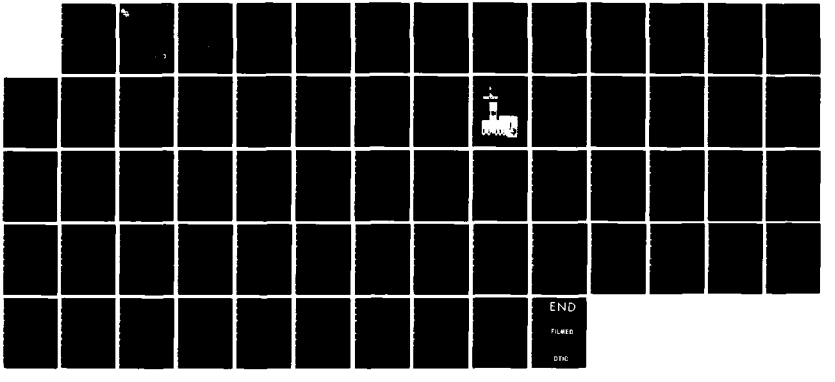

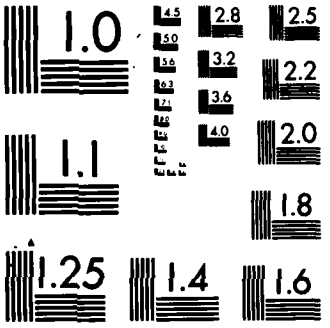

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A MULTI–ISOTOPE PROCEDURE FOR SIMULTANEOUSLY ESTIMATING THE VOLUME OF BODY FLUID COMPARTMENTS OF SWINE

CAROL A. BOSSONE, MA
and
JOHN P. HANNON, PhD

DIVISION OF COMBAT CASUALTY CARE

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PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

A Multi-isotope Procedure for Simultaneously Estimating the Volume of Body Fluid Compartments of Swine--Bossone and Hannon

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

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ABSTRACT

A procedure was developed for simultaneously estimating the volume of all major body fluid compartments. Splenectomized pigs, 20 to 25 kg, with chronically-implanted carotid artery catheters, received a bolus injection (per kg) (1 uCi ^{125}I-albumin, 11.7 uCi ^{51}Cr-EDTA, 0.6 uCi ^{22}Na, and 5.3 uCi $^{3}H_2O$) followed by a 5.2 uCi ml/kg injection of ^{51}Cr-tagged red cells. Plasma samples were obtained at 30, 45, 60, 90, 120 and 150 minutes after injection. Zero time values were determined by least squares regression. Gamma-emitting isotopes were counted at appropriate energy windows while $^{3}H_2O$, after separation from plasma, was counted in a scintillation spectrometer. Red cell volumes averaged 17.2 ml/kg, plasma volume, 66.5 ml/kg, ^{51}Cr-EDTA space, 246 ml/kg, ^{22}Na space, 303 ml/kg, and total body water 639 ml/kg. Estimated lean body mass averaged 822 g/kg and body fat, 178 g/kg. The new procedure was rapid, technically simple, required small amounts of blood and was applicable to conscious animals.

Key Words: body fluid volumes, multi-isotope procedure, swine, conscious.

PREFACE

The research effort reported here served as a thesis submitted by the senior author to the faculty of the Biology Department at San Francisco State University in partial fulfillment of the requirements for a Master of Arts degree in physiology.

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A MULTI-ISOTOPE PROCEDURE FOR SIMULTANEOUSLY ESTIMATING THE VOLUME OF BODY FLUID COMPARTMENTS OF SWINE

The body composition of swine, including the fluid components and techniques for its measurement, evolved in two dissimilar scientific communities. Researchers associated with the commercial meat industry needed better measurements of body composition to evaluate the outcome of animal management procedures directed at improving the economics of animal growth and the quality of meat products produced (1,2,3). Researchers associated with the biomedical community needed better measurements of body composition to minimize between animal variance and to improve their understanding of certain functional phenomena (4,5,6).

Biomedical investigators long ago discovered, for example, that between-animal variance could be reduced by expressing their experimental measurements in terms of body weight. A far better standardization was sometimes realized when measurements directly or indirectly related to energy metabolism were expressed in terms of fat-free mass, i.e. lean-body mass or active-metabolizing mass (5,6,7). Oxygen consumption and cardiac output, for instance, are more highly correlated with lean body mass than with total body mass (5). The interpretation of some functional phenomena, such as the transcellular and transcapillary fluid exchange associated with exercise or hemorrhagic hypotension, required sophisticated examination of body composition, specifically the developement of techniques to study intercompartmental fluid shifts (8,9).

Body Composition Procedures

Two general techniques are used to assess body composition: the direct and indirect. The direct procedures involve determination of gross compositional values by chemical analysis of the carcass. To determine the chemical composition of an animal the carcass is homogenized, and aliquots are measured for water, fat, protein and mineral content (10). Information obtained from such measurements serves as an invaluable base against which other methods, e.g. the indirect, can be compared. While the results obtained by direct analysis of the carcass may represent some of the most reliable baseline work done in the field of body composition, the experimental procedure is often tedious, expensive, and impractical in the case of large animals or man. In addition, when carcass analysis is performed on market animals such as swine, experimental procedures and findings may not be useful or applicable to the biomedical

researcher. Frequently, the analyses are limited to the eviscerated carcass, the portion of greatest commercial interest (11,12,13). Furthermore, these analyses require sacrfice of the experimental animal. The biomedical researcher needs procedures applicable to the intact conscious animal.

Indirect procedures can provide quick, easy, and fairly accurate measurements of body composition in the live animal. Techniques used in these indirect measurements can be divided into two categories. The first category includes noninvasive techniques such as measuring body density (6,7), ultrasonic estimation of backfat thickness (14,15), total body ^{40}K or ^{42}K (16,17), and electric conductivity (18) as well as a variety of other procedures (7) to assess total body composition in terms of mineral, protein, fat, water, and lean body mass. The second category includes the invasive techniques for measuring body composition. Most of these are based on the dilution of tracers substances in a particular body fluid compartment.

Typically, a known amount of the indicator, chemically or isotopically labeled, is injected intravenously or intraarterially; its dilution is measured after complete mixing in the compartment. Evans Blue dye, for example, combines chemically with plasma albumin upon injection. By injecting this dye intravenously plasma and blood volume can be estimated (19). Chemical procedures however are tedious and frought with technical inaccuracies. Consequently, dilution procedures based on radioisotope tracers, which do not present these analytical difficulties, have become increasingly popular. Isotopically labeled indicators are particularly well-suited for animal research because of the simplicity of the techniques, increased sensitivity, and greater accuracy over conventional dilution techniques. A tracer dose of tritiated water, for example, distributes rapidly in the total body water volume and dilution of the injected dose in plasma water can be readily determined with a scintillation spectrometer. Tritiated water has been used extensively to determine total body water, lean body mass and body fat content of domestic animals (20,21). Other isotopes also have been used extensively. These include ^{51}Cr-tagged red cells to measure circulating red cell volume (22,23), ^{125}I-labeled albumin to measure plasma volume (24), and a variety of isotopes used such as ^{36}Cl (25), $Na^{35}SO_4$ (26), ^{82}Br (27) ^{3}H-inulin (28), and ^{51}Cr-labeled ethylendiaminetetraacetic (^{51}Cr-EDTA)(29) to measure extracellular space.

Porcine Body Composition Studies

For many years carcass analysis has been used to assess the body composition of swine (1,2,3,11,12,13); but noninvasive (14,15,16,17,18) and invasive (17,20,21) indirect procedures are being used more frequently. The first use of isotope indicators was

reported by Hansard et al (30) who employed ^{32}P-tagged red cells to measure blood volume. Subsequently, other early investigators used Evans Blue dye (31), ^{131}I (32), and ^{125}I-labeled albumin (33) to measure plasma volume, and ^{51}Cr-labeled red cells (34,35) to measure red cell volume. In most instances only a single indicator was used and blood volume was calculated on the basis of measured plasma or red cell volume and hematocrit. Bush et al (36), were the first to use two indicators, ^{32}P-tagged red cells and ^{59}Fe-labeled plasma protein, for simultaneous measurements of red cell and plasma volumes. Some of these isotopes presented problems. For example, red cells were inefficiently tagged with ^{32}P, as evidenced by in vitro studies reported by Hansard et al (30). The relatively short half-life (8 days) of ^{131}I-labeled albumin limited its use over extended periods of time, and its gamma spectrum has a major energy peak which overlaps that of ^{51}Cr (37). Consequently, difficulties were encountered when attempts were made to use both ^{51}Cr and ^{131}I for simultaneous measurements of red cell and plasma volumes. Red cells tagged with ^{51}Cr, however, offered an excellent procedure for red cell volume measurements because of the efficiency and permanency of labeling (22,23). Experiments soon followed on the simultaneous measurement of blood volume utilizing ^{51}Cr-tagged red cells and a compatible tracer such as T-1824 (38) or ^{125}I-labeled albumin (39); the major gamma energy peak of the latter isotope was far lower than that of ^{51}Cr (37).

Early experiments using chemical indicators to measure total body water in pigs were based on antipyrene dilution (40,47). This indicator often gave inaccurate values because some of the injected antipyrene was bound to plasma proteins and consequently did not distribute in total body water (41). The use of deuterium labeled and tritium labeled water effectively addressed the problem and offered the added advantage of rapid diffusion and complete equilibrium soon after injection. In pigs, Wood and Groves (42) and Flynn et al (43) reported good agreement between deuterium oxide-based total body water values and water values based on desiccation on the same animal. Deuterium measurements, however, presented analytical problems. Tritiated water was more attractive than deuterated water because of the ease in scintillation counting. Furthermore, Kay et al (20) reported good agreement in pigs between total body water measurements obtained by $^{3}H_2O$ dilution and desiccation. Numerous experiments and reviews followed these early measurements and verified the utility of the deuterium and tritium labels in swine (44,45,46).

Few attempts have been made to measure the extracellular fluid volume of pigs. Thiocyanate, a chemical indicator, was first used and reported by Hornicke (47), and then by Setiabudi et al (45). Remy et al (48) were among the first investigators to use isotopically labeled indicators to measure extracellular space in pigs, namely by the dilution of ^{36}Cl, ^{82}Br, $Na_2{}^{35}SO_4$ and ^{140}La-

diethyltetraminopentacetic acid (^{140}DTPA).

Even fewer attempts have been made to use multi-isotope dilution procedures in pigs to measure several body fluid compartments simultaneously (41,48). Yet, many commonly encountered conditions are associated with fluid shifts from one body fluid compartment to another. Following hemorrhage, for example, pigs show a rapid shift of water and solutes from the extravascular to the intravascular space (unpublished observations). To properly evaluate this phenomenon, plasma volume measurements must be made simultaneously with extravascular volume measurements. The latter would include both interstitial and intracellular space estimates. In species other than swine, some efforts have been made to develop multi-isotope dilution procedures to address such evaluation tasks (8,27,39,50). With occasional exceptions (8), the isotopically labeled indicators were not injected simultaneously, and sometimes the measurements of various body fluid compartment extended over days (27,49). These procedures would be of little or no use in the evaluation of rapidly occuring fluid shifts; the fluid volume changes in one compartment would be completed before those in other compartments were even measured. What is needed then is a procedure that will allow simultaneous, and rapid volume assessments of all of the major fluid compartments in the body. The procedure should be applicable to the conscious animal, be technically simple and require only small quantities of blood for its implementation.

Objectives

The purpose of the studies reported in this paper was to develop isotope-based procedures to measure plasma volume, red cell volume, extracellular fluid volume, and total body water individually. Subsequently, techniques would be developed to use a combination of these procedures to measure some or all of the fluid spaces in the pig simultaneously.

Methods

Experimental Animals

Yorkshire-cross pigs, weighing 19 to 27 kg, were obtained from a local pig breeder (Mr. Boswell, Corcoran, Ca.). They were maintained in a common holding pen until they were studied 2 to 4 weeks later. They were fed a commercial ration (Purina Pig Chow, Ralston Purina Co., St. Louis, Mo.) and received water ad libitum.

After an overnight fast each animal was brought to surgery and given a premesthetic intramuscular injection of 0.8 mg/kg atropine sulfate, 2.2 mg/kg ketamine HCl and 2.2 mg/kg xylazine. The animal

Pipette accuracy. Accuracy of the pipettes used in this study was assessed gravimetrically with distilled water and subsequently with blood. In the latter instance, blood density was first obtained by weighing exactly 100 ml in a volumetric flask and using the same blood to determine the blood weight, and calculated volume, delivered by the 0.2 ml and 1.0 ml positive displacement pipettes and the 10 ml calibrated injection syringes.

Body Composition.

The pigs used in the multi-isotope procedure also were utilized to obtain porcine physical characteristics (porcinometry). Animals were measured for various body dimensions as well as backfat thickness, as determined by an Ithaca Ultrasound Scanoprobe^{R} Model 751A and actual ruler measurements. Body measurements included the following: weight, height, as measured from the ground to shoulder; length, measured from poll to the base of the tail; and neck, girth, and belly circumferences. Following euthanasia of the animal, three sites along the back were chosen for measurements for backfat thickness. The first site was located over the fourth rib, the second over the last rib, and the third over the last lumbar vertebra. All sites were taken 5 cm from the spine on the right side of the animal. In measuring backfat thickness with the Scanoprobe^{R} a small dab of oil is placed on the site and read according to manual instructions. Each site is read three times, alternating sites each time. After the ultrasonic measurements were taken backfat thickness was verified by cutting a slice through the backfat at each of the three sites and measuring the actual thickness with a millimeter rule.

In addition, intracellular water was calculated as the difference between total body water and ECW measured by ^{51}Cr-EDTA. Lean body mass (LBM) and body fat (BF) content were calculated from the total body water measurements according to the following equations:

$$\text{LBM}_{(g/kg)} = \frac{\text{TBW}}{0.778}$$

$$\text{BF}_{(g/kg)} = \text{Body Wt} - \text{LBM}$$

where TBW is total body water in g/kg and 0.778 is the water fraction of lean body mass as determined by various investigators (13,43,52).

compared to single injected isotope experiments so that potential errors in the spectral interference ratios could be minimized. Accordingly, dosage (as CPM in dilution samples) was highest for ^{125}I, intermediate for ^{51}Cr and lowest for ^{22}Na. In the final formulation, each animal received; 0.6 uCi/kg ^{22}Na, 1.0 uCi/kg ^{125}I, 5.2 uCi/kg ^{51}Cr-labeled red cells, 11.7 uCi/kg ^{51}Cr-EDTA, and 5.3 uCi/kg $^{3}H_2O$. The ^{51}Cr-labeled red cells were prepared individually as described in the single injection experiment. The remaining isotopes were mixed together in a second injectate with approximately 10 ml of the pigs plasma and brought to a total volume of 15 ml with normal saline. The two injectates so prepared were then infused rapidly into the pig with calibrated syringes. Dilution samples (4 ml blood) were taken at 30, 45, 60, 90, 120, and 150 minutes after injection. Hematocrit values were obtained at each time point. One-milliliter blood aliquots measured with a positive displacement micropipette were transferred to 12 X 75 mm test tubes and diluted with water for the determination of red cell volume. Blood was then centrifuged at 2000 g for 15 minutes. One-milliliter aliquots of plasma were then pipetted and diluted with 1 ml of water in gamma-counting tubes for the determination of extracellular space and plasma volume. The remaining plasma was extracted for tritiated water according to the procedure outlined previously.

In addition to blood and plasma samples, aliquots of the injected dose dilutions also were counted. 1-ml of the ^{51}Cr-labelled red cell injectate was diluted with water to 50 ml. Subsequently 1 ml of this dilution, in triplicate, was counted. One milliliter of the second injectate (containing ^{51}Cr-EDTA, ^{22}Na, ^{125}I, and $^{3}H_2O$) was diluted with water to 1000 ml and triplicate 1-ml aliquots of this dilution were also counted to determine the ^{22}Na and ^{51}Cr-EDTA injected dose. An additional milliliter of this second injectate was diluted with water to 100 ml to determine the ^{125}I concentration in the injected dose. Because the tritiated water had been mixed with the gamma emittors it was necessary to extract aliquots (in triplicate) of the 1:1000 dilution on the extraction apparatus.

Instrument Calibration

Spectrometer accuracy. Standard dilution curves were made for each of the five different isotopes used in the experiments reported here. This was necessary in order to determine the point at which the CPM of a sample became too high to be determined accurately by the spectrometers used in the study. Various dilutions of each isotope were made, and the CPM's were plotted as a function of their respective activities. The resultant curves were analyzed for deviations from the expected linear relationship. In this way, sample CPM could be kept in a range at which the counter could accurately measure activity (uCi).

when ^{51}Cr and ^{125}I were present in the same plasma sample, true CPM for ^{125}I could be determined by the following equation:

$$^{125}I \text{ CPM} = (\text{CPM at } 15\text{–}80 \text{ keV}) - R(\text{CPM at } 240\text{–}400 \text{ keV})$$

It should be noted that spectral energy from ^{125}I, does not impinge on the ^{51}Cr energy window.

In the second experiment, a range of ^{22}Na concentrations was prepared and, ratios of ^{22}Na energy impingement on both the ^{51}Cr (R') and ^{125}I (R") windows were determined. In this, and other experimental work, an energy window of 433 to 1417 keV was used for ^{22}Na counting. When a plasma sample contained all three of the foregoing isotopes true ^{51}Cr CPM were calculated as:

$$^{51}Cr \text{ CPM} = (\text{CPM at } 240\text{–}400 \text{ keV}) - R'(\text{CPM at } 433\text{–}1417 \text{ keV})$$

and true ^{125}I CPM were calculated as:

$$^{125}I \text{ CPM} = (\text{CPM at } 15\text{–}80 \text{ keV}) - R(\text{true } ^{51}Cr \text{ CPM}) - R''(\text{CPM at } 433\text{–}1417 \text{ keV})$$

Simultaneous ^{51}Cr-EDTA and ^{51}Cr-RBC determinations. When a blood sample contains both ^{51}Cr-labelled red blood cells and ^{51}Cr-EDTA in plasma, the plasma contribution must be subtracted from the total blood sample count to determine the ^{51}Cr/ml of red cells. Thus,

$$^{51}Cr/\text{ml RBC} = \frac{(^{51}Cr/\text{ml blood}) - [1 - (0.97)(Hct_a)]\,[^{51}Cr\text{-EDTA/ml plasma}]}{(0.97)(Hct_a)}$$

Multi-isotope injections. To determine different compartmental volumes with the use of a multi-isotope procedure nine animals were first used in preliminary experiments to assess the feasibility of the injections, and to modify the doses if necessary. These animals were each injected simultaneously with 2 to 4 different isotopically labeled indicators in various combinations. Following these preliminary experiments, seven pigs were each injected with a mixture of five different labels. The dosages of each were modified as

evaporation during extraction was minimized by covering the top of each Dewar flask with a 4 X 4 X 3/4-inch square of polyurethane foam, which had a 3/4-inch hole cut in the center (with a cork borer). The foam was slit to the periphery to facilitate its placement around the water collection tube (see Fig. 1).

The rate of water extraction by the apparatus was measured by placing 1-ml plasma aliquots in extraction tubes and periodically measuring the weight loss of these tubes on an analytical balance as extraction progressed. The efficiency of $^{3}H_2O$ extraction, relative to unlabeled H_2O extraction, was measured by placing 5 ml of water of known specific activity ($^{3}H_2O$ CPM/ml of water) in each of a series of extraction tubes. These tubes were then extracted over increasing periods of time such that progressively greater amounts of water, as measured on an analytical balance, were transferred. The specific activity of $^{3}H_2O$ in the extracted samples was subsequently measured and expressed as a percentage of the initial specific activity.

The effectiveness of separating $^{3}H_2O$ from plasma containing gamma emitting isotopes was first assessed by preparing a mixture of ^{125}I, ^{51}Cr and ^{22}Na in water. Three samples (2 ml each) of the mix were placed in extraction tubes, frozen and extracted over a 2-hour period. From each of the extraction samples, 0.5-ml aliquots were counted on the scintillation spectrometer to test for gamma emitter contamination. In the second assessment of effectiveness, tritiated water was added to the ^{125}I-^{51}Cr-^{22}Na mixture after which 0.2 ml samples were added to 2 ml of plasma. These plasma mixtures, in triplicate, along with 2 ml of the initial $^{3}H_2O$ dose, also in triplicate, were extracted for 2 hours, after which 0.5-ml aliquots in 10 ml scintillation fluid were counted on the scintillation spectrometer to assess the efficiency of $^{3}H_2O$ transfer.

Spectral interference. When more than one gamma-emitting isotope is present in a plasma sample spectral energy from one or more isotopes can impinge on the energy windows used for counting the other isotopes. Unless appropriate corrections are made, such energy impingement will seriously compromise counting accuracy. The separation of counts due to one nuclide from those due to another can be accomplished on the Packard Auto Gamma® spectrometer, with its three channel (energy window) analyzer system. Standard samples of the three gamma-emitting isotopes used in these experiments (^{51}Cr, ^{125}I and ^{22}Na) were counted and spectral interferences, expressed as ratios, were determined.

Two experiments were conducted to establish the magnitude of these ratios. In the first, a range of ^{51}Cr concentrations were prepared and counted simultaneously in the peak energy windows for ^{51}Cr (240 to 400 keV) and ^{125}I (15 to 80 keV). Ratios of CPM in the ^{125}I window to those in the ^{51}Cr window were calculated for each ^{51}Cr concentration and an average ratio value (R) was determined. Thus,

vacuum pump. All tubing connections were secured with worm drive tubing clamps. The manifold and central portions of the extraction units were permanently mounted and secured by Castaloy®, 3-pronged clamps to Flexiframe® support. This support also was used to secure the vapor traps.

To use the apparatus for the extraction of plasma water, the following sequence of operations was followed. The plasma samples to be extracted, usually 1 ml, were placed in test tubes which had been filled to a depth of 2 to 4 cm with loosely packed glass wool; the latter was used to prevent foaming when the plasma melted under vacuum. The tubes containing plasma were then attached in turn to left-hand arms of each extraction unit. The stopcocks connecting the units to the manifold were closed, as well as the stopcocks connecting the manifold, to ambient air and the manifold to the first vapor trap. The stopcock between the second vapor trap and the vacuum pump was opened. About 750 ml liquid nitrogen were poured into two 1000-ml Dewar flasks, Pope® type 8645, installed on the vapor traps and secured to the Flexiframe® support with large Castaloy® prong clamps. The vacuum pump was then turned on and the trap portion of the apparatus was evacuated. It was important to follow this sequence of operations since any tritiated water that might have collected in the traps during earlier usage of the apparatus would be frozen before a vacuum was applied. The manifold portion of the apparatus was evacuated next. While all of these operations were going on, the tubes containing plasma samples were being frozen with liquid nitrogen in 265-ml Dewar flasks, Pope type 8600. Wooden blocks sawed from 4 X 4-inch stock were used to support these flasks around the tubes. When freezing was complete, as evidenced by a suppression of liquid nitrogen boiling, the stopcock connecting the first unit to the manifold was opened, and each unit in turn was evacuated. Evacuation usually required about 1 minute per unit to achieve a pressure of 100 mtorr, a convenient end point that was readily monitored with the vacuum gauge. After all of the plasma containing units were frozen, the stopcocks connecting them to the manifold were closed. After a few minutes wait each was reopened to verify maintainance of the vacuum, as determined with the vacuum gauge. When this was verified the stopcocks were again closed and the Dewar flasks under the plasma-containing tubes were removed and placed under the empty tubes of each unit. The vacuum pump was then turned off, the Dewar flasks on the vapor traps were removed, and the manifold was opened to ambient air; vacuum, obviously, still persisted in each of the extraction units. The manifold must not be opened to ambient air while the vapor trap Dewar flasks are still in place since oxygen contained in ambient air will condense in the traps, a potentially hazardous outcome.

As the evacuated plasma-containing tubes gradually rewarm, water vapor is released and subsequently diffuses to the empty test tube of each unit where it is refrozen by liquid nitrogen. Liquid nitrogen

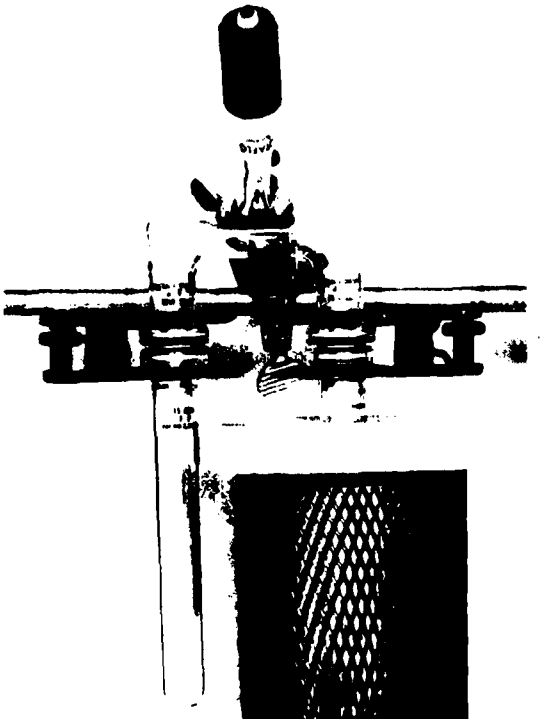
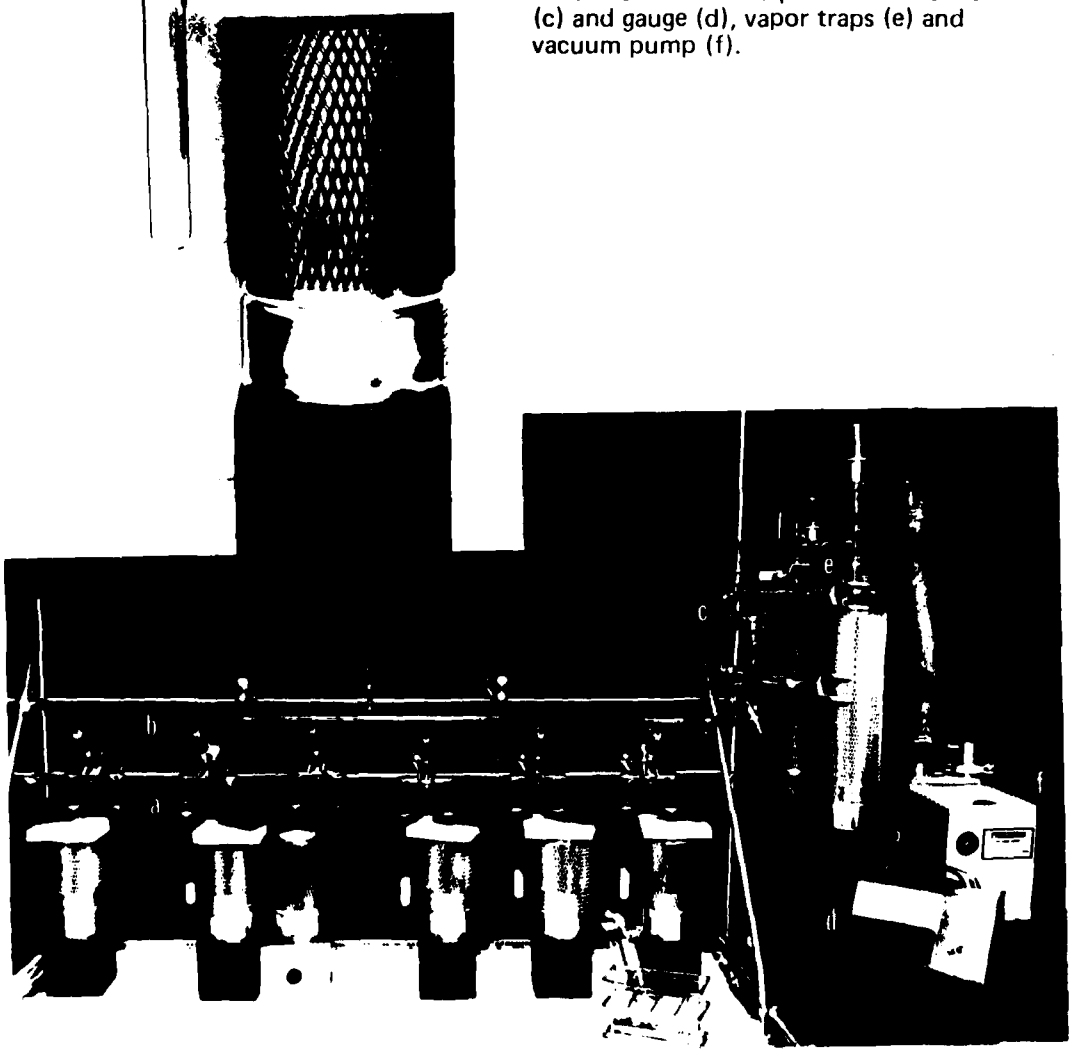


Figure 1 (left). Water extraction unit. Left arm contains glass wool to prevent plasma foaming. Right arm is inserted in Dewar flask covered with foam insulator to minimize liquid nitrogen evaporation.

Figure 2 (below). Water extraction apparatus including 6 extraction units (a), connected by stopcocks to manifold (b), in turn connected to pressure transducer (c) and gauge (d), vapor traps (e) and vacuum pump (f).



where ID is the total CPM in the injected dose and A_o is the zero-time dilution value.

Multi-isotope Dilution Procedure

Tritiated water extraction. When gamma-emitting isotopes are present in a plasma sample, tritiated water, a beta emitter, cannot be counted in scintillation spectrometer, unless the water is extracted and counted separately. To accomplish this task, the apparatus shown in Figures 1 and 2 was constructed in our glass-working shop. The apparatus allows freezing of the plasma sample with liquid nitrogen followed by vacuum extraction of the water contained in the sample and the collection of this water in pure form.

The apparatus consists of six extraction units connected to a manifold which in turn is connected to a high vacuum pump (Figure 2). Six units were used in order to provide a six-point dilution curve for total body water determinations. Each unit (Figure 1) was constructed from two, 15 mm I.D., Lab-Crest®, borosilicate glass joints. One limb of each joint was formed into a test tube, approximately 15 cm in length; these test tubes serve as reservoirs for plasma samples and water extracted therefrom. The other two joint limbs approximately 3.5 cm long were joined to a 15 mm I.D. tube, such that the overall unit, when assembled, formed an inverted "U" with the two test tube portions about 7.5 cm (center-to-center) apart. Thomas No 28 pinch clamps secured the test tubes to the central portion of each unit. The center portion of each unit was joined to the manifold (Figures 1 and 2) with a 6 X 10 mm Corning Rotoflo® stopcock; this connection allowed independent use of each of the six units. The manifold was constructed from 20 mm I.D. borosilicate glass tubing. A 3 X 8 mm Corning Rotoflo® stopcock was installed in the mid portion of the manifold to allow access to ambient air when an evacuated unit was recompressed. One end of the manifold tube was sealed and the other was reduced in size and formed into grooved connector which would accept 6 mm I.D. rubber vacuum tubing. One end of an 8-cm tubing section was attached to the manifold. The other end was attached to a 6 mm glass T connector. One limb of this connector was attached to a 3 X 8 mm Corning Rotoflow® stopcock which in turn was connected by means of vacuum tubing to the transducer of a Hargent-Welch, thermocouple-based, vacuum gauge. The other limb of the T connector was attached by means of vacuum tubing to a 10 X 13 mm Corning Rotoflo® stopcock which had been joined to the inlet of a 48 X 250 mm, freezing-type vapor trap. The outlet of this trap was connected by vacuum tubing to the inlet of a second similar-sized vapor trap. Finally, the outlet of the second trap was joined, through a 10 X 13 mm Corning Rotoflo® joint and 15 mm I.D. tubing to a Precision, Model DD 90,

disappearance, and A_o and k values characterizing this portion were determined. The curve stripping procedure, and determination of A_o and k values, could be repeated as necessary to describe the characteristics of any additional compartments that might be represented by the original indicator-dilution curve.

The water volume of a second compartment (V_2) would be calculated as:

$$V_2 = \frac{(ID)(0.95)}{(A_0 + A_0')(Wt)}$$

where A_o is the zero-time dilution values for indicator dilution in the extracellular space and A'_o is the zero-time value for indicator dilution in the second compartment. Additional compartmental volumes would be calculated by including appropriate A_o values in the denominator.

Total Body Water

Two pigs were used to develop methods for measuring total body water. In the first animal, 30 uCi/kg ^3H_2O in 10 ml of saline was injected intraarterially. This was done rapidly with the calibrated Fisher Varipet® syringe and flushed in with 15 ml of saline. A second pig was injected with 4 uCi/kg of ^3H_2O.

Dilution samples of 3 ml blood were taken at 1-minute intervals for 10 minutes and then at 15, 20, 30, 45, 60, 90, 120, 150, 180, 240, and 300 minutes after injection. Blood was centrifuged at 2000 g for 15 minutes and 0.5-ml aliquots of plasma, measured with the positive displacement pipette, were mixed with 10 ml of scintillation fluid and 0.5 ml of water. The injected dose of ^3H_2O was measured in triplicate by counting 0.5-ml aliquots of duplicate 1:200 dilutions of the injection solution. These 0.5-ml aliquots were each pipetted into 10 ml of scintillation fluid and mixed with 0.5 of ml of plasma. All samples were counted on the Packard scintillation spectrometer with an energy window set at 0-19 keV.

Percent water in plasma was used in total body water volume calculations. The CPMs of the dilution samples were plotted on semilogarithmic paper and the zero-time dilution value was determined using a least-squares regression procedure. Total body water (TBW) as determined by ^3H_2O dilution in plasma was calculated as:

$$TBW(ml/kg) = \frac{(ID)(0.95)}{(A_0)(Wt)}$$

procedure was then applied to the sample CPMs beyond this point to delineate the disappearance kinetics and zero-time dilution value of each isotope label. Other compartmental volume values, in addition to extracellular space, sometimes could be determined by applying curve stripping procedures to indicator dilution values preceding the above determined point of exponential disappearance.

Volume calculations. Extracellular water volume (ECW) was calculated as:

$$ECW(ml/kg) = \frac{(ID)(0.95)}{(A_0)(Wt)}$$

where ID was the total CPM in the injected dose, 0.95 represented the correction factor for percent water in pig plasma, and A_o was the zero time CPM/ml of plasma.

Biological half-life of the extracellular label was determined as:

$$t_{½} = \frac{0.693}{k}$$

Clearance of the isotope from the extracellular compartment was calculated as:

$$C(ml/min/kg) = (ECV)(k)$$

The following general equation was used to determine the contribution of additional compartments to the overall dilution curve of an extracellular volume indicator:

$$A_t = A_0 e^{-kt} + A'_0 e^{-k't} + \cdots$$

where A_t is the CPM/ml plasma at any time t, A_o, A'_o, and k, k' are the zero-time values and the rate constants for each of the compartments contributing to the overall dilution curve. To determine the zero-time and rate constants characterizing each compartment, the A_o and k value for extracellular space was first determined by semilogarithmic least-squares regression as described above. These values were then used to calculate the theoretical dilution values before the point at which exponential decay was actually observed. The theoretical points were subtracted (stripped) from the observed dilution values and the differences so obtained were again plotted on semilogarithmic paper. A second, semilogarithmic, least-squares regression procedure was applied to the portion of the new curve that displayed exponential

minutes after injection as described for red cell volume measurements. Blood was centrifuged at 2000 g for 10 minutes and 0.5-ml aliquots of plasma were pipetted into 10 ml of aqueous counting scintillant (ACS) fluid (Amersham) along with 0.5 ml of water. The injected dose of ^{3}H-inulin was measured in triplicate by counting 0.5-ml aliquots of duplicate, 1:200 dilutions of the injection mixture. These 0.5-ml aliquots were each pipetted into 10 ml of scintillation fluid and mixed with 0.5 ml of an unlabeled plasma blank. Water or plasma additions were necessary to maintain a constant quench factor in the dilution and injected dose samples. All samples were counted on a Packard Tri Carb^{R} scintillation spectrometer, Model 4530, with an energy window set at 0-19 keV.

A third animal was injected with 2 uCi/kg $Na_2{}^{35}SO_4$ diluted in 10 ml of saline. Dilution samples and aliquots of the injection solution were taken and measured as outlined in ^{3}H-inulin experiment with an energy window on the Packard scintillation spectrometer set at 0-170 keV.

A fourth animal was injected with 6 uCi/kg ^{51}Cr-labeled ethylenediaminetetraacetic acid (^{51}Cr-EDTA). Plasma dilution samples were obtained as outlined for ^{3}H-inulin, and 0.5-ml aliquots were transferred to 12 X 75 mm disposable test tubes and diluted to 2 ml with water. These samples along with 0.5-ml aliquots from duplicate 1:200 dilutions of the injection solution were counted on the Packard Auto Gamma^{R} spectrometer with an energy window set at 240-400 keV.

The final two pigs were used to determine the in vivo dilution kinetics of ^{22}NaCl. In one pig 4 uCi/kg and in the other 2 uCi/kg of ^{22}Na diluted in 10 ml of 0.9% saline were injected as described previously. Dilution samples and aliquots of the injection solution were prepared for counting as described in the ^{51}Cr-EDTA experiment. Samples were counted on the Packard Auto Gamma^{R} spectrometer with an energy window set at 433-1417 keV.

Plasma water. In the total body water and extracellular fluid experiments, indicator dilution was calculated in terms of plasma water. The water fraction of porcine plasma was determined by weighing 1 ml plasma samples on an analytical balance. The plasma was then dried in a vacuum oven at 60 degrees Celsius for 24 hours. Plasma was weighed again and the difference between wet weight and dry weight represented the fraction of water in the plasma. Three to five trials were run on six pigs and a mean value was calculated.

Zero-time dilution. As with the blood volume measurements, theoretical zero-time dilution values were calculated on each of the four isotopes used to measure extracellular space. The counts per minute of the dilution samples were thus plotted on semilogarithmic paper to determine the point at which indicator dilution became an exponential function. A least-squares semilogarithmic regression

by ^{51}Cr-labeled cell dilution in the splenectomized pig, was calculated as:

$$RCV_{(ml/kg)} = \frac{(ID)(0.97)(Hct_a)}{(CPM_b)(Wt)}$$

where ID is the CPM in the injected dose, Hct_a is the arterial hematocrit, CPM_b is the counts per minute per milliliter of blood, Wt is body weight in kg, and 0.97 is a correction factor for trapped plasma.

Plasma volume, as determined by ^{125}I, was calculated as:

$$PV_{(ml/kg)} = \frac{ID}{(CPM_p)(Wt)}$$

where CPM_p is the calculated, zero-time counts per minute per ml of plasma after dilution.

Total blood volume (TBV) was calculated as:

$$TBV_{(ml/kg)} = RCV + PV$$

In addition, total body hematocrit (Hct_b) and the ratio of Hct_b to Hct_a, commonly termed F_{cells}, were calculated as:

$$Hct_b = \frac{RCV}{RCV + PV}$$

$$F_{cells} = \frac{Hct_b}{Hct_a}$$

Extracellular Volume Measurements

Six pigs were used to determine the in vivo dilution kinetics of four isotopically labeled indicators commonly used for measurements of extracellular space. Two animals were injected intraarterially with 10 ml of approximately 6 uCi/kg ^{3}H-inulin in normal saline, and the injectate was flushed in with 20 ml of saline. Dilution samples of 3 ml of blood were taken at 1-minute intervals for 10 minutes and then at 15, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 330, and 360

Trapped plasma. Hematocrit values used in the calculation of erythrocyte and plasma volumes were corrected for trapped plasma as determined by a procedure similar to that reported by Baker (51). Briefly, an accurate, gravimetrically measured volume (30 to 70 ml) of porcine blood was placed in a beaker along with an accurately measured dose of ^{125}I-bovine albumin or $Na_2{}^{35}SO_4$. The blood and isotope were gently, but thoroughly, mixed for 10 minutes with a magnetic stirrer, at the end of which 5 or more microhematocrit capillary tubes were filled and centrifuged at 12,000 g for 5 minutes. The remaining blood was centrifuged at 2000 g for 10 minutes, plasma was collected and the activity of ^{125}I-bovine albumin or $Na_2{}^{35}SO_4$ was determined in 5 or more 1 ml plasma aliquots. Average isotope dilution was used to calculate the beaker plasma volume and, by difference from the original beaker blood volume, the beaker erythrocyte volume and hematocrit. Finally, the difference between beaker hematocrit and the slightly lower average capillary tube hematocrit was used to calculate the plasma fraction trapped in the erythrocyte column packed by the microhematocrit centrifuge.

Plasma volume. A second pig was used to develop techniques and appropriate dosages for measuring plasma volume by the dilution of ^{125}I-labeled bovine albumin. Accordingly, 1.0 uCi/kg ^{125}I-albumin was mixed with 10 ml of the pig's plasma. This mixture was brought to a total volume of 15 ml with 0.9% saline. Ten milliliters of this mix was injected intraarterially into the pig and dilution samples were taken as outlined in the ^{51}Cr-labeled red cell experiment. The whole blood was centrifuged at 2000 g for 15 minutes, after which 1.0 ml plasma aliquots were transferred along with 1 ml of water to 12 X 75 mm test tubes. Triplicate 0.2 ml aliquots of the injected dose were similarly transferred and diluted. The Packard Auto Gamma® spectrometer with energy windows set at 15-80 keV was used for counting.

Zero-time dilution. Since ^{51}Cr-labeled red cells are slowly lost and ^{125}I-labeled albumin is more rapidly lost from the circulating blood, it was necessary to calculate theoretical, zero-time dilution values. This was accomplished by first plotting CPM in the dilution samples on semilogarithmic paper (with time as the linear coordinate) and ascertaining the time point at which dilution became an exponential function. Zero-time dilution was then determined by a semilogarithmic, least-squares regression procedure applied to the dilution samples beyond this point.

Volume calculations. Red cell volume (RCV), as determined

resuspended, washed, and centrifuged three times with 10 ml of ice-cold physiological saline. After each centrifugation, 1 ml of saline wash was transferred to 12 X 75 mm test tubes and diluted with 1 ml of water; these samples were used to measure ^{51}Cr losses in the saline washes. Positive displacement micropipettes (Scientific Manufacturing Industries, Emeryville, Ca.) which were independently calibrated were used for all sampl transfers. The sample counts per minute (CPM) were determined with a Packard Auto Gamma® spectrometer, Model 500C, with an energy window set at 240-400 keV. CPM values obtained with this spectrometer, and in subsequent measurements with a scintillation spectrometer were corrected for background radiation. The following series of calculations were used to determine red cell labeling efficiency (L.E.):

$$\text{Initial } ^{51}Cr \text{ CPM in blood} = (28.7)(50)(\text{CPM/1ml aliquot})$$

$$\text{CPM in plasma} = (1 - \text{Hct})(28.7)(10)(\text{CPM/1ml aliquot})$$

$$\text{CPM in each saline wash} = (10)(\text{CPM/1ml aliquot})$$

$$\text{L.E.}(\%) = \frac{\text{CPM in blood} - \text{CPM in plasma and washes}}{\text{CPM in blood}} \times 10^2$$

Red cell volume. One pig was used to determine the approximate dosage and dilution kinetics of autologous ^{51}Cr-labeled red cells that would be appropriate for the measurement of circulating red cell volume. A $Na_2{}^{51}CrO_4$ dose of [illegible] uCi/kg was thus used to label the red cells. However, only two saline washes were employed, and the labeled cells were ultimately mixed with 10 ml of saline. A 10-ml aliquot of this mixture was then carefully measured with a calibrated Fisher Varipet® syringe and injected intraarterially into the pig and flushed in with 20 ml of 0.9% saline. Dilution samples, 3 ml blood, were taken at one-minute intervals for ten minutes and then at 15, 20, 30, 45, 60, 90, 120, 150, 180, 240 and 300 minutes after injection. To assure acquisition of fresh circulating blood, sample removal was immediately preceded by a 15-second period of continuous blood withdrawal from the catheter at a rate of about 1 ml/sec. After sample removal, this blood was returned to the animal and the catheter was filled with a minimal volume, approximately 1 ml, of heparinized saline (10 units/ml). A 1 ml blood aliquot, measured with the positive displacement micropipette, was taken from each sample, transferred to a 12 X 75 mm disposable test tube and diluted with 1.0 of ml water. The hematocrit of each blood sample also was determined. In addition to the dilution samples, triplicate, 0.2 ml aliquots of the original ^{51}Cr-labeled red cell mixture were placed in 12 X 75 mm test tubes and diluted with 2.0 ml of water. These aliquots also were transferred with a calibrated positive displacement micropipettes. All samples were counted as described above.

was then anesthetized with halothane in oxygen and nitrous oxide induced by a face mask and maintained with an endotracheal tube. To simplify red cell volume measurements the animal was first splenectomized according to procedures described elsewhere (39). Subsequently, the left carotid artery was exposed surgically and a 1.27 mm I.D. X 2.03 mm O.D. polyvinylidine catheter (Type S-54 H.L. Tygon, Norton Plastics and Synthetics, Akron, Oh.) was inserted and advanced to the level of the aorta. The catheter was held in place with circumvascular ligatures; the free end was tunneled beneath the skin and exteriorized on the dorsal surface of the neck. Then the end was fitted with a 17-gauge Intramedic® Luer stub adapter (Clay Adams, Parsippany, N.J.) and closed with an Argyle® intermittent infusion plug (Brunswick Co., St. Louis, Mo.). The catheter was flushed with 1 ml heparin (1000 units/ml) and a Velcro® (Velcro USA New York, N.Y.) patch with a 2 cm X 7.5 cm hole in the portion nearest the skin was sutured over the exited end of the catheter. The animal was then allowed to recover 7 to 10 days.

On the day of the study the pig was brought to the laboratory in a portable holding cage and given waste paper as bedding material. The catheter line was opened and connected to a one foot pressure monitoring/injection line (Cobe Laboratories, Lakewood, Co.) with a 3-way stopcock (Pharmaseal Inc., Toa Alta, Puerto Rico). The catheter was cleared and flushed with fresh heparin/saline mix solution. The animal was allowed to become comfortable and usually assumed a recumbent position. After 30 minutes of voluntary recumbent rest the experiment began.

Blood Volume Measurements

Red cell labeling. Red cell volume was determined by the dilution of ^{51}Cr-labeled red cells. Labeling procedures similar to those described by Nelson and Swan (23). In a preliminary in vitro experiment, labeling efficiency was determined by mixing 160 uCi of $Na_2{}^{51}CrO_4$ (in 0.7 ml physiological saline), with 25 ml of freshly collected porcine blood and 3 ml of citrate/phosphate/dextrose (CPD) solution (total volume = 28.7 ml). The hematocrit of this mixture was determined with a Lourdes microhematocrit centrifuge (Vernitron Medical Products Inc., Carlstadt, N.J.). To determine total radioactivity, a 1-ml sample was removed and mixed with 50 ml of water from which triplicate 1 ml aliquots were transferred to 12 X 75 mm disposable test tubes and diluted with 1 ml of water. The residual blood was then allowed to stand for 30 minutes at room temperature with occasional mixing to facilitate red cell labeling. Subsequently, the blood was centrifuged at 2000 *g* and plasma collected. A 1-ml plasma sample was diluted to 10 ml with water and triplicate 1-ml aliquots were transferred to 12 X 75 mm test tubes and diluted with 1 of ml water; this sample was used to measure the amount of ^{51}Cr remaining in plasma. The red cell pellet was

Data analysis.

Pigs utilized in these experiments were analyzed as groups depending on which body fluid compartments had been measured. These included the animals from the single isotope experiments, the nine pigs used in preliminary multi-isotope injection experiments as well as the seven pigs injected simultaneously with the five isotopic labels. Group means, standard deviations, and standard errors of the mean were obtained for all measured data.

RESULTS

Instrument Calibration

The standard curve relating ^{51}Cr CPM as a function of uCi present in the counting sample showed that deviations from linearity occurred when sample radioactivity exceeded about 250,000 CPM (Figure 3). Similar curves also were constructed for ^{35}S, ^{22}Na, ^{125}I, and ^{3}H. Deviations from linearity varied from isotope to isotope, for example from 100,000 to 250,000 CPM. In all of the experiments reported in this paper, consequently, sample CPMs were kept below the deviation points, generally from 1000 to 50,000 CPM.

The results of trials testing the accuracy of the pipettes and the 10-ml injection syringe are presented in Table 1. A high degree of accuracy was obtained with the positive displacement pipettes and the injection syringe. Furthermore, no significant differences were determined between the measured volumes of water and blood.

Blood Volume Measurements

Red cell labeling. Table 2 summarizes data on the efficiency of the ^{51}Cr-labeling as practiced in the experiments reported here. The total efficiency after 30 minutes of incubation at room temperature was 83.6%. Most of the residual ^{51}Cr (14.7%) was removed with the plasma following the first centrifugation. Negligible contamination was observed after the second wash and centrifugation. Consequently, only two washes were used when ^{51}Cr-labeled cells were prepared for red cell volume measurements.

Red cell volume. After injection of ^{51}Cr-labeled red cells into a splenectomized pig, dilution samples as illustrated in Figure 4, were plotted over a 45 minute post-injection period. Values obtained during the first minute or two after injection were higher than those recorded subsequently. These elevations and subsequent cycling of the CPM could be due to recirculation of the labeled cells close to the point of injection and sampling. Complete mixing of the labeled cells, however, occurred within 10 minutes and a zero-time dilution

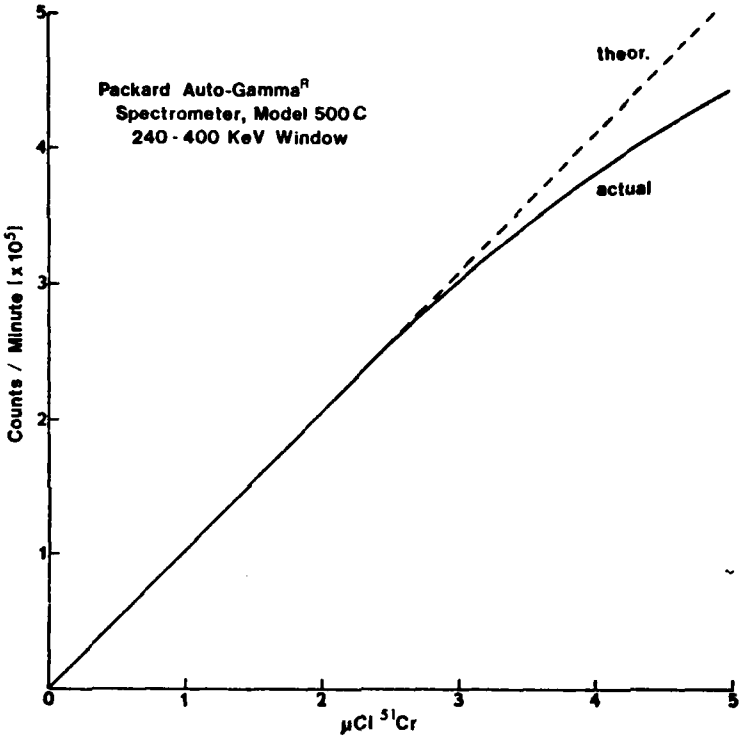


Figure 3. Actual and theoretical ^{51}Cr counting accuracy at increasing radioactivity levels.

TABLE 1. Measurement accuracy of pipettes and injection syringe

	Positive Displacement Pipette				Calibrated Syringe	
	0.2 ml		1.0 ml		10 ml	
Trial *	H_2O	Blood	H_2O	Blood	H_2O	Blood
1	0.1989	0.2000	0.9951	0.9993	9.9915	9.9906
2	0.1996	0.1968	1.0040	1.0033	10.0078	10.0253
3	0.2002	0.1974	0.9964	1.0004	10.0041	9.9990
4	0.1994	0.1999	0.9978	0.9930	9.9935	9.9836
5	0.1993	0.1993	1.0048	0.9968	9.9985	10.0070
mean	0.1995	0.1987	0.9996	0.9986	9.9991	10.0011
S.D.	± 0.0005	± 0.0007	± 0.0020	±0.0017	± 0.0031	± 0.0072

*Each trial value, in ml, represents the average of triplicate measurements of distilled water or blood. Replacement 0.2 and 1.0 plastic tips and different 10 ml syringes were used for each trial.

TABLE 2. Efficiency of ^{51}Cr-labeling of Porcine red cells

Sample	Total CPM	% Total CPM
Blood (initial)	24,667,650	100
Plasma	3,627,456	14.71
First Wash	308,380	1.25
Second Wash	79,780	0.32
Third Wash	27,810	0.11
	Labeling Efficiency =	83.6

Abbreviation: CPM, counts per minute.

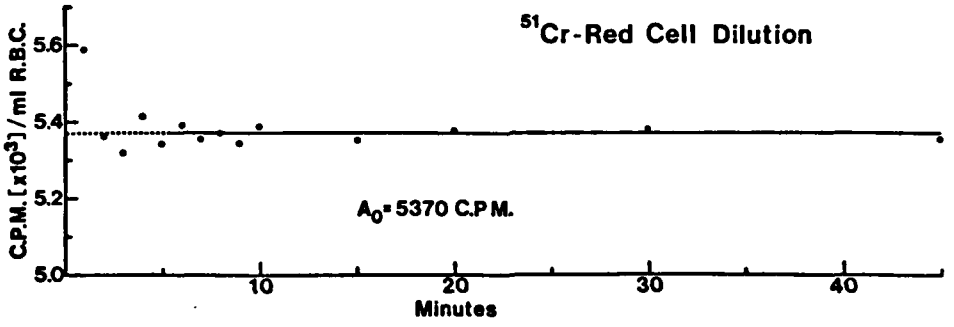


Figure 4. Dilution of autologous ^{51}Cr-labeled red cells in the arterial blood of a conscious splenectomized pig.

value, A_o, was readily calculated. A total erythrocyte volume of 24.7 ml/kg was obtained for this animal.

Trapped plasma. The results of the trapped plasma determination are shown in Table 3. There was no significant difference between the values based on ^{125}I-albumin and $^{35}SO_4$ dilutions. The values obtained in the 12 trials ranged from 1.2 to 5.0% for a composite average of 2.8 ± 1.26%.

Plasma volume. The plasma ^{125}I-albumin dilution curve for a splenectomized pig is illustrated in Figure 5. Points were plotted over a 160-minute period after injection. During the first 10 minutes, only the 2, 5, and 10-minute values are shown although samples were obtained and counted at one minute intervals. Evaluation of the dilution kinetics of this radiolabel revealed two distribution components. The first became apparent about 2 minutes after injection and was characterized by rapid disappearance of the label as evidenced by the short half-life of 4.87 minutes. This earlier component appeared to represent plasma mixing and circulatory recycling of the label close to the point of injection. The more slowly disappearing component became apparent about 20 minutes after injection and had a half-life of 4.67 hours. Disappearance, presumably, was due to ^{125}I-albumin loss to the interstitial space. The plasma volume estimates reported below were based on the least-squares extrapolation of this dilution component to zero-time.

Extracellular Volume Measurements

Plasma water content. Table 4 shows the water content in plasma obtained from six different pigs. The values ranged from 94.0 to 95.0% and the average value (95%) was used in all of the calculations of extracellular and total body water volumes.

Dilution kinetics. The dilution kinetics of ^{22}NaCl, $Na_2{}^{35}SO_4$, ^{51}Cr-EDTA, and ^{3}H-inulin are presented in Figures 6, 7, and 8. Figure 6 shows the ^{22}Na CPM plotted over 6 hours. The rapid decrements in ^{22}Na activity observed in the first 30 minutes were presumably due to loss of the isotope from the plasma to the interstitial space. Complete mixing, as evidenced by the monoexponential rate of loss from the extracellular fluid space, was achieved at 30 to 60 minutes after injection.

Figure 7 illustrates the comparative plasma dilution characteristics of $Na_2{}^{35}SO_4$ and ^{51}Cr-EDTA. To facilitate this comparison the CPM/ml plasma was normalized for both curves by assigning 100 to the 10-minute values. The early mixing component for both labels was similar to that of ^{22}NaCl; equilibration in the extracellular space was established at about 30 to 60 minutes after injection. Kinetic evaluation of the dilution values beyond 30 to 60

TABLE 3. Trapped plasma (T.P.) in erythrocyte column after microhematocrit centrifugation.

	^{125}I-albumin			$Na_2{}^{35}SO_4$	
Trial*	Hct	T.P. (%)	Trial*	Hct	T.P. (%)
1	0.22	3.2	7	0.26	2.0
2	0.28	2.7	8	0.33	2.2
3	0.29	2.3	9	0.30	4.7
4	0.30	1.2	10	0.28	1.2
5	0.30	2.1	11	0.33	4.1
6	0.29	5.0	12	0.29	3.4
mean	0.28	2.7		0.30	2.9
S.D.	± 0.030	± 1.28		± 0.028	± 1.35

*Blood from separate pigs (N = 12).

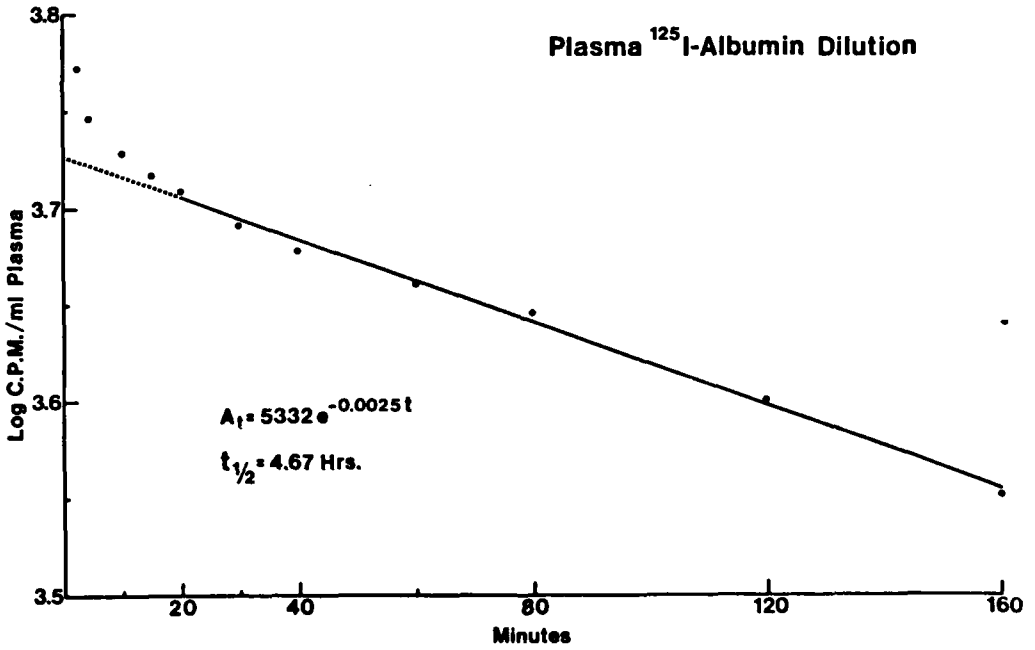


Figure 5. Dilution of ^{125}I-labeled bovine serum albumin in the arterial plasma of a conscious splenectomized pig.

TABLE 4. Water Content of Porcine Plasma Samples[a]

Pig	Initial Weight	Final Weight	Difference	% H_2O
3	1.4634	0.5078	0.9556	95.6
4	1.4711	0.5196	0.9515	95.1
10	1.4749	0.5291	0.9458	94.6
16	1.4851	0.5451	0.9400	94.0
17	1.4950	0.5361	0.9589	95.9
19	1.4805	0.5319	0.9486	94.9
			Average =	95.0

[a]Values for each pig represent averages obtained from triplicate 1 ml plasma samples. Initial and final weights include weighing of pan.

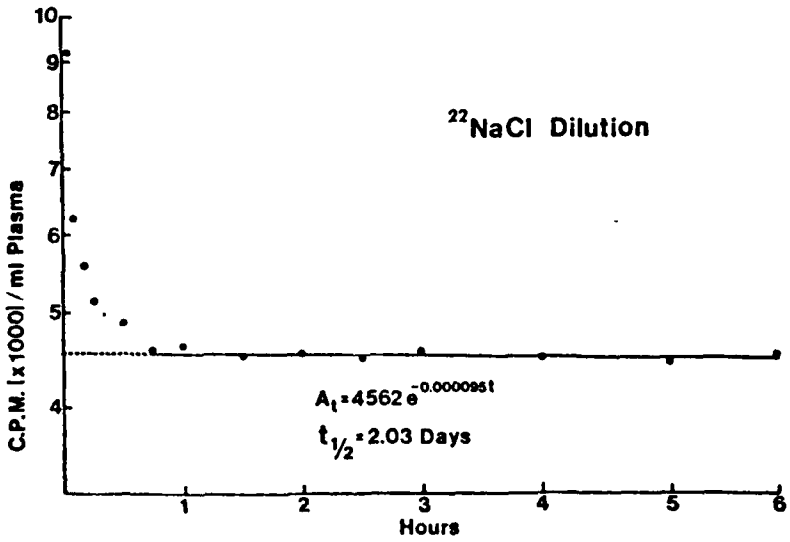


Figure 6. Dilution of ^{22}NaCl in the arterial plasma of a conscious splenectomized pig.

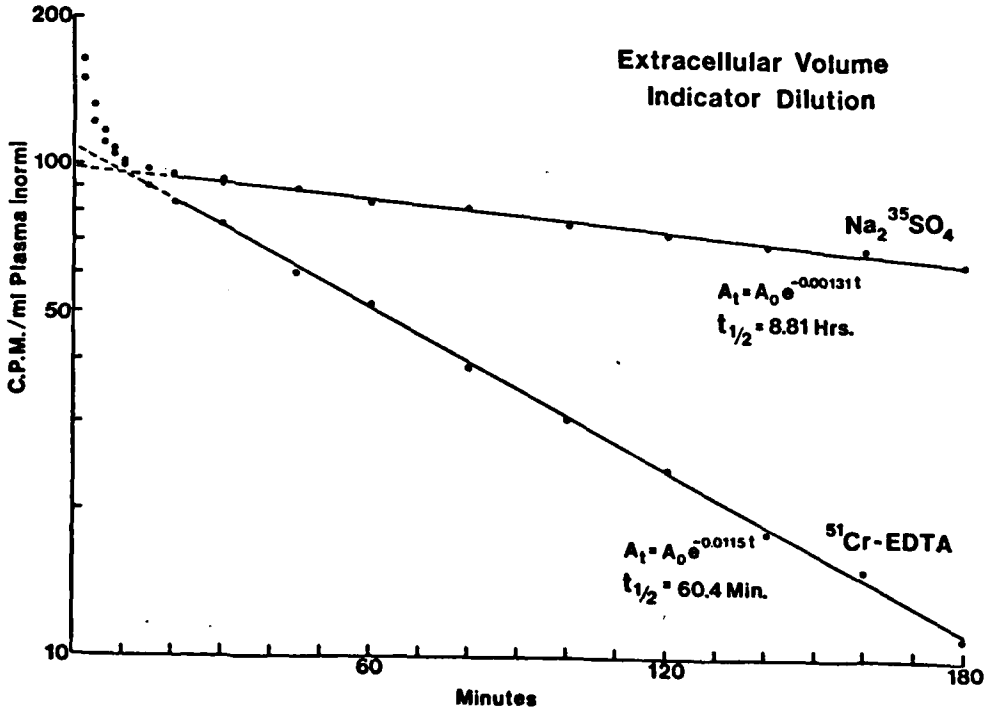


Figure 7. Comparative dilution characteristics of $Na_2{}^{35}SO_4$ and ^{51}Cr-EDTA in the arterial plasma of conscious pigs.

minutes showed that $Na_2{}^{35}SO_4$ had a slower renal clearance than ^{51}Cr-EDTA. This is shown by the longer half-life of $Na_2{}^{35}SO_4$ (8.81 hours) when compared to that of ^{51}Cr-EDTA (60.4 minutes).[2] Despite the difference in disappearance kinetics, similar extracellular volume values were obtained with the two radiolabels. A value of 163 ml/kg was obtained by using $Na_2{}^{35}SO_4$ and 174 ml/kg was obtained with ^{51}Cr-EDTA.

Of the four isotopes used to measure extracellular space, tritiated inulin showed the slowest equilibration, presumably because of its larger molecular weight. The more complex circulatory and interstitial mixing characteristics of this indicator allowed the estimation of at least 3 different fluid compartments (Figure 8). Kinetic evaluation of the dilution curve was accomplished by curve stripping and least-squares regression procedures. Extracellular volume was based on zero-time extrapolation of the final monoexponential dilution curve which was assumed to reflect complete mixing. This curve appeared at about 90 minutes after injection, and had a t 1/2 of 100 minutes, not too different from the half-life of ^{51}Cr-EDTA dilution (Figure 7). The estimated extracellular volume based on these data was 212 ml/kg. An extracellular subcompartment, revealed by the kinetic analyses had a volume of 102 ml/kg; the anatomical nature of this compartment is unknown. Another extracellular subcompartment, plasma volume, can be estimated by kinetic analyses. The value so estimated was 51.8 ml/kg. Half-life of the ^3H-inulin in plasma (2.97 minutes) was so short, however, that accuracy of this volume estimate could be questioned.

Total Body Water

Figure 9 shows the semilogarithmic plot of ^3H_2O dilution in plasma. In this pig tritium was equilibrated in total body water in about 30 minutes. Beyond this point ^3H_2O water disappearance was slow as indicated by the long half-life of 1.78 days. The calculated total body water for this animal was 667 ml/kg.

Multi-isotope Measurements

Tritiated water extraction. The results obtained from measurements of the rate of water extraction with the extraction apparatus are illustrated in Figure 10a. Total extraction (both labeled and unlabeled) was achieved after 2 to 3 hours at a pressure in the extraction units of 100 mtorr. This rate, as it progressed, varied possibly because of differences in water distribution throughout the glass wool at the bottom of the extraction tube. The rate also was extended markedly when the vacuum was reduced or lost due to an insufficient seal of the O-rings in the extraction unit joints. With reasonable care of operation, this problem was not encountered often. When it did occur it was usually due to worn

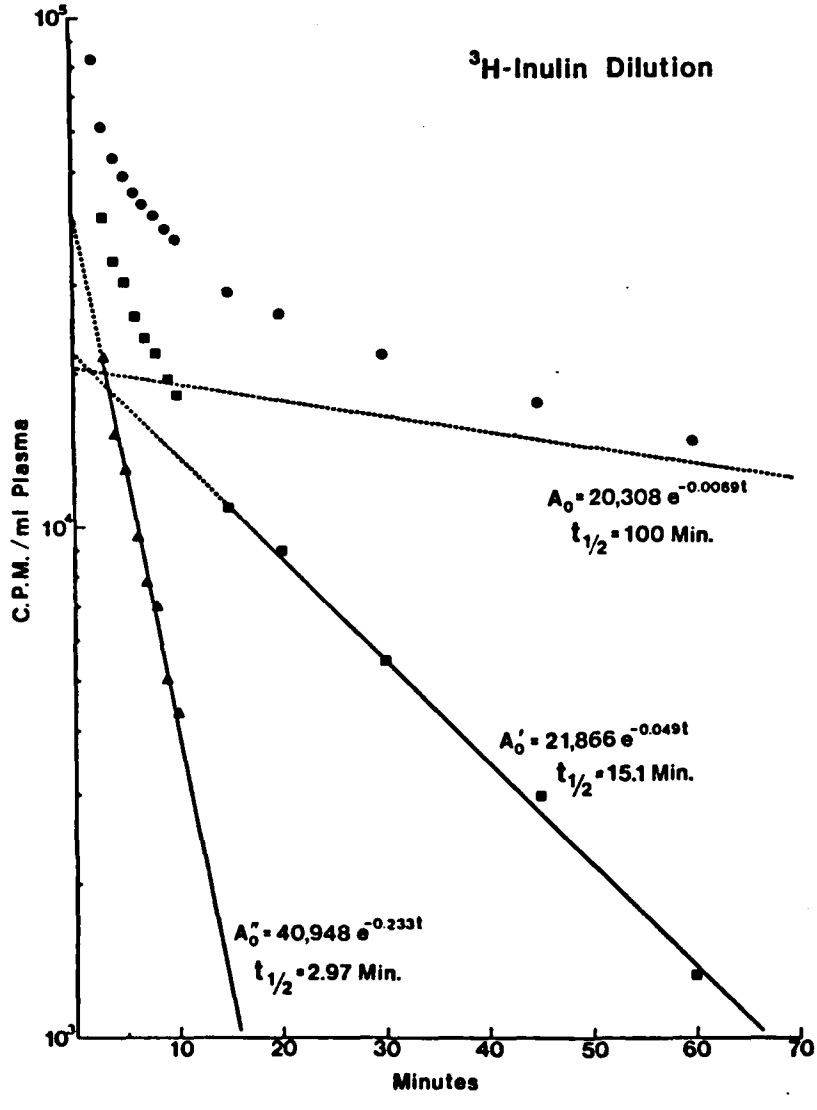


Figure 8 In vivo dilution of ^{3}H-inulin in the arterial plasma of a conscious splenectomized pig. Upper curve was extrapolated from dilution values extending out to 300 minutes. Lower two curves were plotted on the basis of curve stripping techniques (see text).

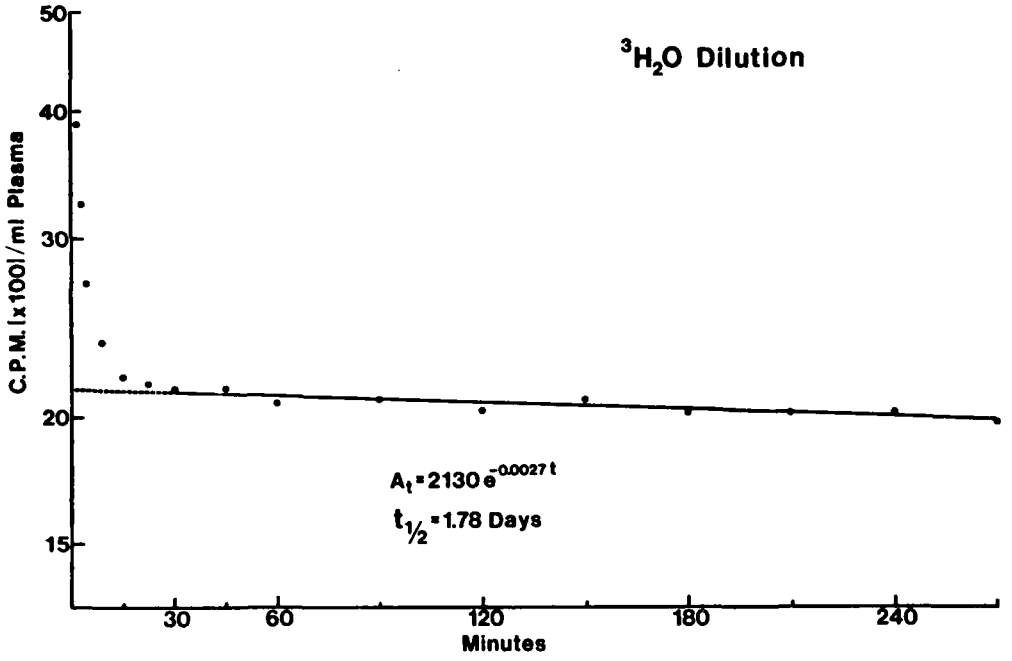


Figure 9. Dilution of ^3H_2O in the arterial plasma of a conscious domestic pig.

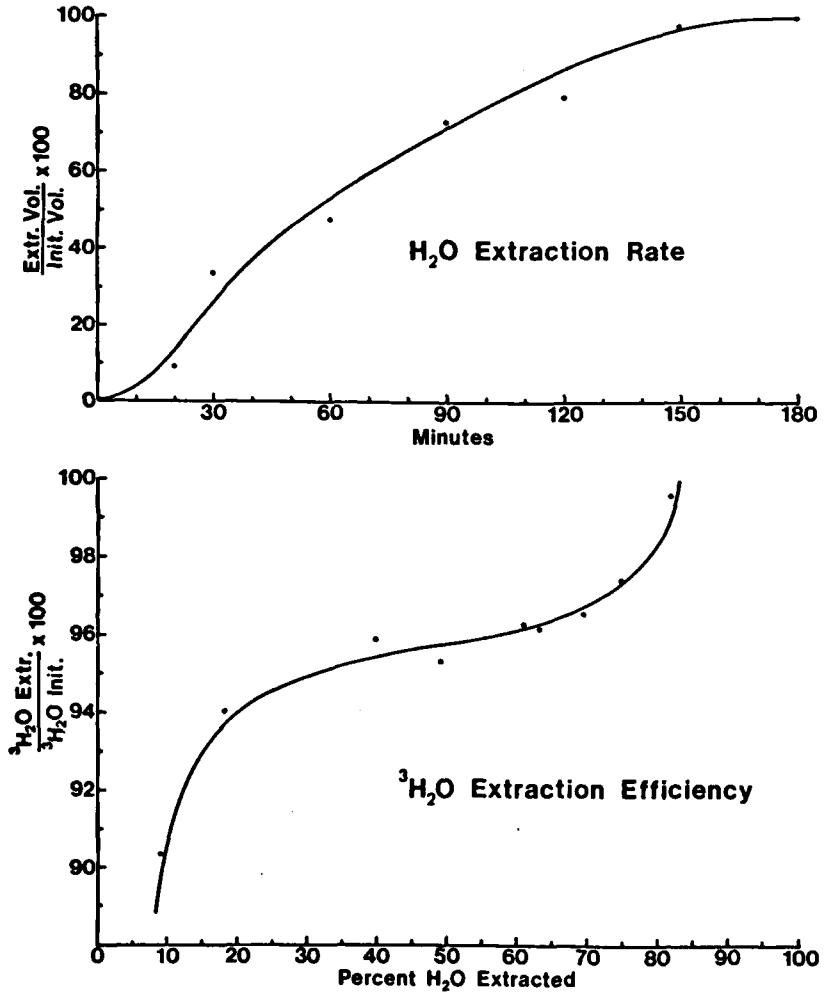


Figure 10a (upper). Rate of water extraction from a one ml plasma sample with water extraction apparatus.

Figure 10b (lower). Efficiency of $^{3}H_2O$ extraction with the water extraction apparatus.

O-rings or strands of glass wool extending over the O-ring joint.

Extraction efficiency, or the percent of ^{3}H transferred relative to that present in the initial tritiated water sample, was calculated as a function of the total water transferred. The results of these calculations are shown in Figure 10b; the tritiated water was extracted at a slower rate than unlabeled water. The $^{3}H_2O$ fraction increased rapidly as the first 10% or so of total water was extracted. Thereafter, a semi-plateau was observed, (between 30 and 70% total water extracted) after which another rapid increase was observed.

The effectiveness of separating the $^{3}H_2O$ from plasma containing a mixture of the gamma-emitting isotopes was evaluated in three separate experiments. In the first experiment, a plasma aliquot deliberately contaminated with ^{51}Cr (1706 gamma CPM) was added to scintillation fluid and counted again with the beta-counting spectrometer. This spectrometer recorded 10314 CPM. Thus, minimal gamma contamination seriously compromised the accuracy of $^{3}H_2O$ counting. In the second experiment, the water extraction apparatus was used to separate $^{3}H_2O$ from a mixture containing approximately 2400 CPM of ^{22}Na, 18,000 CPM of ^{51}Cr, and 4800 CPM of ^{125}I as determined by the gamma-counting spectrometer. The extracted water, counted with the scintillation (beta-counting) spectrometer, showed essentially background activity (less than 150 CPM). In the third experiment, a mixture containing (per ml) 13,940 beta CPM of $^{3}H_2O$, 3800 gamma CPM ^{22}Na, and 3000 gamma CPM ^{125}I was prepared and the water was extracted and counted on the beta-counting spectrometer; 13,880 CPM/ml were recorded in the latter instrument. Thus, the last two of these experiments showed that the water extraction apparatus was highly effective in separating $^{3}H_2O$ from plasma containing gamma-emitting isotopes.

Spectral interference. Table 5 contains the results of tests designed to assess the magnitude of spectral energy impingement from ^{51}Cr into the counting window used for ^{125}I. Except at low ^{51}Cr levels, a reasonably constant (average 0.093) ratio of CPM in the ^{125}I window to CPM in the ^{51}Cr window was obtained. Deviations at low ^{51}Cr levels could be due to variations in background radiation recorded in the ^{125}I window.

Results of tests to evaluate the magnitude of ^{22}Na energy impingement into the ^{51}Cr and ^{125}I windows are included in Table 6. Again, reasonably constant ratios were obtained if the counts in the ^{51}Cr and ^{125}I windows did not approach background levels. If these deviations were ignored, the ratio of CPM in the ^{51}Cr window to CPM in the ^{22}Na window averaged 0.051. The ratio of CPM in the ^{125}I window to CPM in the ^{22}Na window averaged 0.108. These ratios, and the ratio for ^{51}Cr impingement in the ^{125}I window (Table 5) were used in multi-isotope studies to calculate true values for ^{51}Cr and ^{125}I.

TABLE 5. Contamination of the ^{125}I Energy Window by Spectral Energy from ^{51}Cr

^{51}Cr (μCi)	^{51}Cr (CPM)	^{125}I (CPM)	^{125}I:^{51}Cr
0.585	84,521	7,726	0.091
0.351	50,733	4,712	0.093
0.234	34,460	3,272	0.095
0.117	16,934	1,572	0.093
0.070	10,209	948	0.093
0.035	5,077	467	0.092
0.012	1,669	177	0.106*
			Average = 0.093

^{51}Cr was counted in its primary window, 240-400 keV, and in the ^{125}I primary window, 15-80 keV. All counts were corrected for background radiation.

*Value not included in calculation of average.

TABLE 6. Contamination of ^{51}Cr and ^{125}I Energy Windows by Spectral Energy from ^{22}Na

^{22}Na (μCi)	^{22}Na (CPM)	^{51}Cr (CPM)	^{51}Cr:^{22}Na	^{125}I (CPM)	^{125}I:^{22}Na
0.065	96,397	10,324	0.107	4,937	0.051
0.030	44,090	4,607	0.104	2,272	0.051
0.020	29,396	3,143	0.107	1,477	0.050
0.010	14,732	1,559	0.106	766	0.052
0.005	7,114	810	0.114	362	0.051
0.0024	4,023	416	0.103	193	0.048*
0.0014	2,335	253	0.108	96	0.041*
0.00035	646	74	0.114	29	0.045*
			Average = 0.108		Average = 0.051

^{22}Na was counted in its primary window, 433-1417 keV, the ^{51}Cr primary window, 240-400 keV and in the ^{125}I primary window, 15-80 keV. All counts were corrected for background radiation.

*Value not included in calculation of average.

Body Composition

Porcinometry. The physical measurements of 12 pigs used throughout the series of studies are presented in Table 7. The backfat thickness values were measured with a millimeter rule at the three sites indicated in the methods. Backfat thickness measured ultrasonically at the same sites yielded essentially equal values. Between-animal variations in height, length, and circumferences appeared to be related to differences in body weight.

Dilution kinetics. The dilution for 5 radiolabeled indicators, $^{3}H_2O$, $^{22}NaCl$, ^{51}Cr-EDTA, ^{51}Cr-labeled red cells, and ^{125}I-albumin after the simultaneous injection into a pig are presented in Figure 11. The 6 dilution samples for each isotope were plotted to 150 minutes after injection. In several cases, particularly at the earlier time intervals points were deleted as is apparent in the dilution curves for ^{51}Cr-EDTA, $^{3}H_2O$, and $^{22}NaCl$ (Figure 11). These points, invariably, were located above the exponential curve.

Blood volume. The blood volume results obtained from simultaneous multi-isotope dilution experiments on 8 pigs are presented in Table 8. Except for the plasma and blood volume values, between-animal variance was relatively low. Plasma volume values ranged from a low of 47.0 to a high of 73.0 ml/kg and blood volume from a low of 66.6 to a high of 92.1 ml/kg. Constancy of the red cell volume measurements was particularly notable, especially when compared to the plasma volume measurements. The body hemotocrit was considerably lower than the arterial hematocrit, and this difference led to an F_{cell} ratio that averaged 0.70.

Water, fat, and lean body mass. Table 9 contains data from 9 pigs for which total body water, $^{22}NaCl$ and ^{51}Cr-EDTA extracellular space, and renal ^{51}Cr-EDTA clearance were simultaneously determined. The results showed a significantly ($p<0.05$ by paired t test) higher volume for $^{22}NaCl$ distribution than the volume of ^{51}Cr-EDTA distribution. The intracellular space values were calculated as the difference between total body water volume and extracellular volume as estimated by ^{51}Cr-EDTA dilution. Two additional pigs were included in computing the average values for total body water, $^{22}NaCl$ space, body fat, and lean body mass. Between-animal variance, in relative terms, was lowest for total body water and lean body mass measurements and highest for ^{51}Cr-EDTA space, ^{51}Cr-EDTA clearance, intracellular water, and body fat measurements.

measurements and calculations. Few procedures reported in the scientific literature incorporate all of these attributes.

RECOMMENDATIONS

- The effects of age on the volume of porcine body fluid compartments should be investigated with the procedure reported here.
- An accurate procedure for estimating the plasma volume of swine should be developed.
- Multivariate regression equations, based on simple measurements, should be developed to predict the lean body mass and body fat content of swine.

TABLE 10. Body Fat, Lean Body Mass, Total Body Water and the Water Percent of Lean Body Mass as Determined by Carcass Analysis of 15 to 30 kg Pigs.

N	Body Fat (g/kg)	Lean Body Mass (g/kg)	Total Body Water (g/kg)	Total Body Water (% L.B.M)	Ref.
		15-20 kg Pigs			
6	189 ± 14.9	811 ± 14.9	634 ± 13.7	78.1 ± 1.33	52
2	188 ± 25.6	812 ± 2.25	634 ± 29.0	78.1 ± 1.13	42
		20-25 kg Pigs			
2	171 ± 14.1	829 ± 3.0	644 ± 12.7	77.7 ± 1.41	52
1	119 ± ...	881 ± ...	695 ± ...	77.1 ± ...	43
8	113 ± ...	887 ± ...	693 ± ...	79.4 ± ...	13
1	158 ± ...	842 ± ...	652 ± ...	77.4 ± ...	42
		25-30 kg Pigs			
2	178 ± 7.1	822 ± 7.1	640 ± 1.40	77.9 ± 0.60	52
1	102 ± ...	898 ± ...	693 ± ...	77.1 ± ...	43
Ave.	152	847	661	77.8	

In some instances (ref. 13,43) carcass values were measured after removal of gastrointestinal contents. In others (ref. 42,43,52) values for water percent of lean body mass were calculated from data presented by the authors.

Standard deviations could not be determined in all instances.

plasma dilution samples.

Body composition. The division of the body into fat and lean compartments has been recognized as advantageous when considering such factors as growth or energy metabolism. The term "lean body mass", first proposed by Behnke (6), more closely represents the active metabolizing cell mass than body weight. Doornenbal and his colleagues (34,84) observed a high correlation between total blood volume and red cell mass, the oxygen carriers, and lean body mass, the oxygen consumer. However, when lean body mass, or body fat are determined on the basis of values measured by dilution procedures certain assumptions must be made. These assumptions presume a constancy of lean body mass. One "constant" often questioned is that lean body mass has a uniform proportion of water. This was first studied by Pace and Rathbun (4) who used carcass analysis to measure the water and fat content of various tissues of the guinea pig. These investigators obtained a value of 73.2 as the percent water in lean mass. This value can vary, depending on the species and size of the animals. In pigs, the water fraction of lean body mass ranges from 85% for newborns to 51% for 145-kg hogs (13,42,43,52). This variation is due to compositional changes in animals throughout growth. Shields et al (13) and Doornenbal (11) measured total body water, total fat, protein and ash on pigs from birth to 145 kg. They (11,13) found that percentage of fat increased curvilinearly while percentage of water and ash declined curvilinearly as body weight increased. A review of changes in body water and fat content throughout various stages of growth in pigs has been compiled in Sheng and Huggins (21). In the present study, the water fraction of lean body mass was assumed to be 0.778 (Table 10). This value was the average of values calculated from data presented by various investigators (13,42,43,52) for 15 to 30 kg pigs.

CONCLUSIONS

The experimental studies described in preceding pages demonstrate development evaluation, and utilization of a simultaneous multi-isotope injection procedure for estimating the volume of various body fluid compartments. Red cells labeled with ^{51}Cr were used to measure circulating red cell volume, ^{125}I-labeled bovine albumin to measure plasma volume, ^{51}Cr-EDTA, and ^{22}Na to measure extracellular volume, and $^{2}H_2O$ to measure total body water. In addition, this procedure allowed calculation of a number of other anatomic variables including interstitial volume, intracellular volume, lean body mass and total body fat. The attractive features of this procedure include its ready applicability to a conscious animal, the injection of all indicators (except ^{51}Cr-labeled red cells) as a single bolus, improved accuracy because of multiple dilution samples, small amount of blood (4 ml), short time to complete the dilution measurements, and simple methods for making

reported by Wood and Groves (52) and Hansard (78). Wood and Groves (42) also reported good agreement between deuterium and dessication in pigs ranging in size from 0.88 kg to 22 kg. In a study of larger pigs (97 kg), Clawson et al (62) compared antipyrene values with total body water determined by carcass analysis. For the 9 pigs in their study, the antipyrene values were greater than direct chemical analysis values. Studies performed on rabbits by Moore (76) and Pace et al (79) measured total body water with D_2O or tritium followed by desiccation and showed that both isotopes provided reasonably accurate estimates of total body water. Pace et al (79) reported that tritiated water could be used as an estimate of total body water with an error comparable to that of D_2O (10%). Sheng and Huggins (80) also measured total body water first with tritium and then by desiccation in beagles and observed an overestimation by the isotope procedure. These investigators attributed the difference to the exchange of ^3H with other molecules of the body such as fat, protein, and carbohydrate. In addition, they speculated that another source of error in the measurement of total body water with tritium could occur in tissues in which water tightly bound and, as a consequence, would not exchange readily with deuterium or tritium. Such impediments to exchange would lead to an underestimation of total body water. Additional sources of errors such as possible exchange of water with the environment or nonuniform isotope specific activity due to slow mixing are discussed in studies by Nagy and Costa (81), Tisavipat et al (82) and Coleman et al (75).

Water extraction. Finally, accuracy of total body water measurements by ^3H_2O dilution are dependent on the accuracy with which the label can be measured in the plasma dilution samples. The major errors in this regard are attributable to the quenching effects of plasma, hence improved accuracy is achieved when plasma water is separated, and ^3H_2O counted, independent of other plasma constituents. Two techniques have been described for the separation of tritiated water from plasma. The more complicated of these, as described by Pierson et al (54) involves combustion of the plasma sample in an oxygen atmosphere with subsequent trapping of the water vapor that is evolved. A simpler procedure, first described by Vaughan et al (83), involves vacuum sublimation. The procedure described in the present study represents an improvement of the latter technique. It allows simultaneous extraction of 6 samples, which can be individually manipulated, vacuum level can be monitored to detect leaks and the apparatus incorporates vapor traps to prevent losses of tritium to the environment. One minor problem was encountered in the use of this apparatus: fractionation of tritiated water occurred when samples were not taken to near dryness. The problem, potentially, could lead to a 3 or 4% error (overestimation) of total body water determinations if only 20 to 70% of the water in plasma samples was extracted. Such errors can be eliminated by extracting the samples to dryness. The errors can be minimized (cancelled) by identical extraction of the injected dose mixture and

compared with the results of the present study a similar renal clearance rate was observed.

Some indicators such as inulin, sucrose, and EDTA have the disadvantage of rapid urinary excretion. Inulin, in addition, has a long equilibration period because of its large molecular size. ^{51}Cr-EDTA although rapidly excreted is a popular volume marker at the present time because it quickly distributes itself in the functional extracellular space, does not enter cells, does not react chemically with any body constituent, and is easily measured in plasma samples (29).

Problems arise when one attempts to calculate intracellular space on the basis of total body water and extracellular water measurements. Since total body water indicators diffuse through all water compartments, including transcellular water, while extracellular volume indicators only diffuse in the interstitial space, the difference between total body water and extracellular volume will overestimate true intracellular space (73).

Total body water. A variety of dilution procedures utilizing $^{3}H_2O$, D_2O and labeled and unlabeled pyrazolones have illustrated the ease with which total body water can be measured (41,75,76). All of these indicators readily diffuse through all the water of the body, including transcellular water, and attain equilibrium fairly rapidly. Antipyrene and its labeled and unlabeled derivatives have been used in several studies of pigs (20,40,41,47,77), and the reported results are in close agreement with values obtained by other methods (desication or isotopic water labels). The rapid in vivo elimination of the antipyrene and its binding to plasma protein represent unfavorable characteristics (20). For this reason D_2O and $^{3}H_2O$ have become more popular indicators for measurements of total body water. Comparisons between tritium and deuterium distribution volumes have shown good agreement as was discussed in a review by Sheng and Huggins (21). The ease and accuracy of scintillation counting has made tritium a more favorable choice for studies in animals, and the absence of radiation has made deuterium a more favorable choice for studies in humans.

Some questions have been raised about space actually measured since these isotopes can be exchanged with hydrogen in metabolic processes, such exchange leading to an overestimation of total body water (68). In pigs, however, studies of deuterium or tritium dilution versus desiccation, especially in the same animal, have shown reasonable comparable estimates of total body water. Flynn et al (43) and Wood and Groves (42) used deuterium oxide to indirectly estimate total body water after which some of the pigs chosen at random were desiccated. Flynn et al (43) reported a mean difference of +0.4% (D_2O dilution space greater than desiccation). Their absolute volume values agreed with the desiccation values of pigs

vessels, including arterioles, capillaries, and venules (51,65,66). Indeed, direct estimation of small vessel hematocrits has yielded values ranging down to lower than 0.1 (67). In humans and dogs such reduced values in small vessels potentially could account for total body to large vessel hematocrit ratios of about 0.90, a commonly calculated value (65,66).

Blood volume. In addition to the foregoing measurement difficulties, porcine blood volume estimates are also influenced by body size. For example, Hansard et al (30) recorded values that ranged from 70 ml/kg in 3.6 kg piglets to 44 ml/kg in 156-kg hogs. The variations with respect to body weight have been reviewed by Steinhardt et al (60). However Doornenbal et al (34) showed that blood volume of swine tends to be a constant fraction of lean body mass.

Extracellular volume. Extracellular fluid space, anatomically, represents that portion to the body fluids outside of cells. The space can be divided into 3 compartments: plasma, interstitial fluid space and transcellular fluid. An in-depth description of extracellular space and problems inherent in its measurement are discussed by Elkinton and Danowski (68). Boundaries of these fluid spaces tend to be ill-defined and sometimes are difficult to measure accurately, e.g. plasma volume. Common transcellular entities such as the gastrointestinal tract, spinal fluid spaces, serous and synovial cavities, upper and lower urinary tracts, and bile can be defined. By contrast, interstitial space contains a variety of subcompartments (lymph, bone, and cartilage matrices, etc), some containing readily exchangeable fluid and others containing fluid that has a slow turnover time. This disparity makes determination of extracellular volume difficult since the volume of distribution depends largely upon the particular characteristics of the indicator. Most of the commonly used extracellular volume indicators do not distribute to transcellular water (68). Thus they do not equilibrate in the total extracellular space. Some dilution indicators tend to distribute thoughout the entire interstitial fluid space while others tend to be confined to the readily exchangeable space, commonly termed functional extracellular fluid. The former category includes thiocyanate (69), sodium (56,59,69), chloride (25,69,70,71), and bromine (53,71). The latter includes sulfate (26,59), sucrose (54), inulin (28,29), and EDTA (29,72). Some indicators tend to overestimate extracellular volume. Thus, sodium, chloride, bromide, and thiocyanate enter cells (69,71,73); sodium exchanges with bone sodium (56); and sulfate enters organic chemical reactions (74). Kulwich et al (74) observed, for example, that most of the labeled sulfur administered orally was rapidly absorbed and then excreted slowly by the renal pathway. By the fourth day, they (74) reported about 62% of the dose excreted has been in the urine. Their analysis of the intestinal tract contents showed that about half of the ^{35}S was in organic form. When the results of this study (74) were

pig. Total body water volume as determined with $^{3}H_2O$ varied depending on the size of the pigs used. The values ranged from 886 ml/kg (44) and 786 ml/kg (45) in 1 to 12-week old pigs to 590 ml/kg in 60 kg animals (48).

Critique of Methods

Circulating red cell volume. Numerous studies have used the dilution of ^{51}Cr-labeled red cells for measuring circulating red cell volume and have consistently demonstrated the fundamental accuracy of the procedure. Attractiveness of the procedure is attributable in part to a high labeling efficiency. In the present study, 83.6% of the radiolabel was bound to porcine red cells after 30 minutes incubation at room temperature. Such efficiency offers a distinct advantage over other labels such as ^{32}P which in porcine studies by Hansard et al (30) required a 2 to 4-hour incubation period at 37°C to attain 24% labeling efficiency. As shown in splenectomized animals studied in the present report, equilibration of the ^{51}Cr-red cells in the circulation is complete in a few minutes. Furthermore, ^{51}Cr is tightly bound and the biological half-life of the labeled red cells closely approximates that of the red blood cell (23). In contrast, less tightly bound ^{32}P is lost from red cells at about 5 % per hour (30). One complication is encountered when ^{51}Cr-labeled cells are used to measure the red cell volume of animals. In the intact pig the spleen will sequester the labeled cells and replace them with unlabeled cells. This process leads to a rapid decline in the circulating concentration of labeled red cells, a process that may continue for an hour or more before the ratio of unlabeled to labeled cells becomes equal in the circulating blood and spleen (37). As a consequence, circulating red cell volume in the intact pig will be overestimated unless appropriate corrections are made (37). This problem is significant only in animals with a highly contractile spleen including pigs (37), dogs (63) or cats (64).

Plasma volume. Plasma volume measurements based on indicators which bind to plasma proteins present various problems. These indicators tend to overestimate plasma volume since plasma proteins will readily pass from plasma to the interstitial compartment (24, 51, 59). An additional problem is illustrated by the F_{cell} ratio (0.70) obtained in this study. This ratio implies that 30% of the total body plasma volume is not mixed with red cells, an unlikely implication. When the total body plasma volumes of the pigs used in this study were calculated on an assumed total body to arterial hematocrit ratio of 0.90, plasma volume averaged 46.3 ml/kg. Total blood volume, the sum of red cell and recalculated plasma volumes, would then average 63.5 ml/kg, a value considerably below the 82.3 ml/kg obtained when plasma volume was estimated by ^{125}I-albumin dilution. Total body to large vessel hematocrit ratios (F_{cells}) below 1.0 are commonly attributed to relative plasma excess in small

Porcine Body Fluid Volumes

Blood volume. Blood volume measurements reported in the present study agree closely with values reported in an earlier study at this Institute in which the same isotopes, ^{51}Cr and ^{125}I (37) were used (39). The red cell, plasma, and blood volume measurements reported in the earlier study on splenectomized pigs were 18.4 ml/kg, 60.7 ml/kg, and 79 ml/kg respectively. The F_{cell} ratio was 0.756. In the present study, red cell volume was 17.2 ml/kg, plasma volume 66.5 ml/kg, blood volume 82.3 ml/kg, and F_{cells} 0.70. The values observed here also were compared to values reported by other investigators for pigs of similar size. Published red cell volumes tend to exceed those seen in the present study while plasma volumes are comparable (60). Factors contributing to the red cell discrepancy are considered later in the discussion. The F_{cell} ratios observed here and in an earlier study (37), are slightly greater than those reported in 6-week-old pigs by Talbot and Swenson (31) and lower than the values for newborn pigs by Deavers et al (32) and Linderkamp et al (61) and for newborn to 12-week- old pigs by Setiabudi et al (38).

Extracellular fluid volume. The different extracellular volume indicators also were compared with those used by other investigators (45,47,48). The distribution volume of ^{22}Na (303 ml/kg) measured in the present study most closely resembled the distribution volumes determined with thiocyanate by Setiabudi et al (45) and Hornicke (7). Thiocyanate space in the study by Hornicke (47) averaged approximately 300 ml/kg for the 20 kg pigs and the study of Setiabudi et al (45) averaged approximately 350 ml/kg for 4 week old pigs. The extracellular marker ^{140}La-DTPA used in the study of Remy et al (48) had an average distribution volume of 200 ml/kg. The ^{82}Br and $^{35}SO_4$ spaces calculated by Remy et al (48) averaged 255 ml/kg and 211 ml/kg, respectively. These measurements agree favorably with the value obtained from ^{51}Cr-EDTA in the present study, namely 246 ml/kg for pigs of comparable weight.

Total body water. In the present study of pigs, 17.7 to 24.1 kg total body water averaged 639 ml/kg. Indirect measurements of total body water in the live animal reported by other investigators based on the dilution of antipyrene (20,40,41,47,62), D_2O (42,43), or ^3H_2O (41,44,45,48) yielded similar values. Kay et al (20), for example, measured simultaneously total body water in 27 kg pigs with 4-aminoantipyrene (4-AA) and tritium. A value of 563 ml/kg was obtained with ^3H_2O and a value of 609 ml/kg was obtained with 4-AA. In 39 to 42 kg pigs, Hansard et al (20) reported an average total body water of 673 ml/kg measured by antipyrene dilution, 638 ml/kg measured by ^{131}I-antipyrene dilution, and 641 ml/kg measured by ^3H_2O dilution. Wood and Groves (42) measured deuterium dilution in pigs of varying weights. For two pigs (15.9 and 16.5 kg), they determined a volume of 666 ml/kg and, for one pig (22.2 kg), a volume of 639 ml/kg. Flynn et al (43) reported a value of 685 ml/kg for a 21.7-kg

^{24}Na and ^{43}K losses. The procedures for segregation ^{24}Na from ^{43}K radioactivity were not specified. In the second report, Pierson et al (59) measured total body water as the 3-2 hour distribution of $^{3}H_2O$ and extracellular water with $Na_2{}^{35}SO_4$ as the zero-time extrapolates of five dilution samples taken 60 to 180 minutes after injection. Subsequently, $^{24}NaCl$ was injected and, at 18 to 20 hours after injection, dilution samples were obtained and corrected for ^{24}Na urinary losses.

A procedure described by McMurrey et al (27) was even more complex. On the morning of the first day they injected D_2O followed by a ^{51}Cr-tagged red cell and Evans Blue injection 2 hours later after a deuterium equilibration sample had been drawn. Dilution samples were taken at 5, 20, and 40 minutes after injection of the ^{51}Cr-labeled red cells and Evans Blue. The 40-minute sample was also used to obtain a second deuterium dilution sample. In the evening of the first day, ^{82}Br was injected and permitted to equilibrate for 14-hours, to 8:00 AM of the next d ~ at which point a dilution sample was collected. The injected dose was corrected for urinary and stool losses during the 14 hour interval. Subsequent to removing the ^{82}Br dilution sample, ^{24}Na and ^{42}K were injected and permitted to equilibrate for 24 hours (third day). A dilution sample was then obtained and distribution volumes, corrected for urinary and stool losses, were calculated. Radioactivity of ^{82}Br and ^{24}Na were separated spectrally by means of a "fixed interval double counting" method which applied a differential decay procedure based on the half-lives of ^{24}Na and ^{82}Br. In addition to being tedious, these experiments encountered a greater possibility of error due to potential daily fluid fluctuations.

Because of the complexities of the foregoing procedures Shires et al (8) attempted to measure plasma volume, red cell volume, and extracellular fluid volume after a single intravenous injection of three isotopic labels followed by a single venous blood sample taken 20 minutes after injection. They used ^{131}I-albumin ^{51}Cr-labeled red cells and $Na_2{}^{35}SO_4$ as volume indicators. The beta emitter $Na_2{}^{35}SO_4$ was separated from the gamma emitters (^{51}Cr and ^{131}I) by a procedure utilizing a shielded scintillation chamber. The effectiveness of this technique was not stated. Differentiation of ^{131}I CPM from the ^{51}Cr CPM was accomplished by a procedure similar to that used in the present study. Although their procedure (8) described attempts to measure a beta emitter in conjunction with two gamma emitters, a better method was still needed to separate the beta emitter(s) from any gamma emitters present. However, because of the marked overlap of ^{131}I and ^{51}Cr spectral energies, the accuracy of the differentiation procedure could be compromised. More important, the use of a single, 20-minute dilution sample afforded no allowances for indicator loss subsequent to injection. Consequently, apparent dilution volumes would be overestimated, particularly those based on ^{131}I and ^{35}S.

differential gamma spectroscopy. After storage for 1 to 3 weeks in a frozen state to allow for decay of ^{82}Br the specimens were combusted in an oxygen atmosphere, and the derivatives $^{3}H_2O$ and $^{14}CO_2$ were trapped in appropriate systems and counted in dioxane and ethanolamineptoluene scintillator systems, respectively, by liquid scintillation counting. The combustion flask was modified by addition of a silver wool plug to remove ^{125}I from the air stream before collection. The success of this separation was established by subsequent counting of the liquid scintillation vials for gamma photons.

In sheep, Figueras et al (55) administered simultaneous intravenous injections of $^{3}H_2O$, ^{131}I-labeled antipyrene, 4-amino-antipyrene, and ^{14}C-labeled N-acetyl-4-amino-antipyrene to estimate total body and extracellular water volumes. Dilution volumes were based on zero-time values calculated by least squares regression of the monoexponential disappearance curves; the number of dilution points was not specified. The beta-emitting isotopes, ^{14}C and ^{3}H, contained in dilution samples were counted after decay of the gamma emitter, ^{131}I. In view of the half-life of ^{131}I (8.05 days), considerable delay must have been encountered in determining ^{14}C and ^{3}H activities.

Several studies in dogs have employed multiple dilution indicators to measure the volume of various body fluid compartments (56-58). Insofar as can be determined, however, only Levitt and Gaudino (56) used a simultaneous injection procedure. They (56) used D_2O to estimate the volume of body water, and ^{24}Na to estimate extracellular water or ^{42}K to measure intracellular water. One blood sample taken at 1 or 2 hours after injection was used to estimate D_2O dilution. Dilutions of ^{24}Na were measured at 3 hours and ^{42}K at 9 hours after injection. In calculating volumes of distribution, the injected doses were corrected for urinary losses of each indicator.

In man, studies of total body water, measured with $^{3}H_2O$ or D_2O, often included measurements of extracellular water or blood volume (27,50,54,59). In most of these studies the indicators were not injected simultaneously but over several hours (50,59) or days (27). Two reports (50,59) describe a schedule in which both $^{3}H_2O$ and $Na_2{}^{35}SO_4$ were injected on the morning of the experiment followed by an injection of $^{24}NaCl$ or ^{43}KCl 3 hours later. This schedule allowed the beta emitters to be counted before the gamma emitter was injected. Accordingly, Burke and Staddon (50) first injected $^{3}H_2O$ and $^{35}SO_4$ and withdrew dilution samples at 15 minute intervals over a 3 hour period after injection. Dilution volumes were determined by plotting points on a semilogarithmic graph and extrapolating back to determine the equilibrium point at zero-time. Upon completion of this procedure, they (50) injected $^{24}NaCl$ and ^{43}KCl and they measured dilution values over the next two days. The latter required 24 and 48-hour urine collection and correction of the injected doses for

DISCUSSION

Simultaneous Multi-isotope Measurements

Except for the simultaneous use of red cell (^{51}Cr, ^{59}Fe, ^{32}P) and plasma (^{125}I, ^{131}I, Evans Blue) labels, multi-isotope procedures have rarely been used in the assessment of body fluid volumes in swine. The various techniques used in measurements of porcine blood volume and their limitations are discussed in an earlier report (39).

Insofar as can be determined, only three reports contain data on the simultaneous determination of extracellular and total body water volumes of swine. Hornicke (47) used the dilutions of thiocyanate and antipyrene, both measured chemically, for the two volume estimates. He injected the indicators intravenously and distribution volumes were based on zero-time extrapolation of 6 to 8 plasma dilution samples taken over a 3-hour period after the injection. In the second report, Setiabudi et al (45) measured the volume of total body water with $^{3}H_2O$ and extracellular volume with thiocyanate. Plasma $^{3}H_2O$ activity and thiocyanate concentration were estimated from a semilogarithmic plots (presumably not by a least squares technique). In the third report, Remy et al (48) measured plasma, extracellular and total body water in one series of pigs following the simultaneous intravenous injection of Evans Blue (determined chemically), $^{3}H_2O$, and ^{36}Cl. The distribution volumes of the first two indicators were based on zero-time extrapolations of plasma concentrations measured at 60, 90, 120, and 180 minutes after injection. Dilution of ^{36}Cl was measured 24-hours after injection, corrected for urine losses. Over a period of days subsequent to the measurements, dilution of ^{140}La DTPA was used in some of the pigs as an alternate method for estimating extracellular volume. In a second series of pigs Remy et al (48) determined total body and extracellular water on the basis of $^{3}H_2O$ and $^{35}SO_4$ dilution, both indicators being injected simultaneously. On the day following these measurements, ^{82}Br dilution was measured in a manner similar to that employed for ^{36}Cl dilution. ^{82}Br was also used in the study of Maksoud and Kieffer (53) in 4-day-old piglets. In this study ^{82}Br space was measured in control piglets and piglets subjected to surgical trauma. The kinetics of this indicator were studied over a period of 130 minutes in both conditions. These investigators made no attempt to use simultaneous injections of two gamma emitters, presumably because the radioactivities of the different isotopes could not be segregated.

Pierson et al (54), in studies of rats administered 50 uCi of tritiated water intravenously. At 10 time intervals from 5 minutes to 28 hours before death, a mixture of ^{14}C-sucrose and $Na^{82}Br$ was given intravenously. Ten minutes prior to death ^{125}I-labeled albumin was given. Dilution samples were counted for ^{82}Br and ^{125}I by

TABLE 9. Multi-isotope Determination of Body Water Distribution, Body Fat and Lean Body Mass

Pig	Extracellular Water (ml/kg) ^{22}Na	^{51}Cr-EDTA	C_{EDTA}	T.B.W. (ml/kg)	I.C.W. (ml/kg)	B.F. (g/kg)	L.B.M. (g/kg)
15	299	—	—	603	—	225	775
18	314	—	—	653	—	161	839
16	297	251	1.51	653	402	161	839
17	313	267	1.52	648	381	167	833
19	330	285	1.99	610	325	216	784
20	297	233	2.33	652	419	162	838
21	306	307	2.36	613	366	212	788
22	294	192	1.25	672	480	136	864
23	298	232	1.86	665	433	145	855
24	286	221	1.77	627	406	194	806
25	297	211	1.60	639	428	179	821
mean	303	246	1.80	639	398	178	822
S.D.	12.7	39.0	0.378	23.2	54.1	29.8	29.8

Abbreviations: TBW, total body water; ICW, intracellular water; BF, body fat; LBM, lean body mass; C_{EDTA}, renal clearance of ^{51}Cr-EDTA in ml/kg/min.

TABLE 7. Physical Measurements of Immature Domestic Swine

	Range	Mean	S.D.
Body Weight (kg)	17.7 – 24.1	21.3	1.91
Shoulder Height (cm)	40.0 – 46.0	43.6	2.05
Poll-Tail Length (cm)	63.5 – 81.0	72.4	4.82
Neck Circum (cm)	51.0 – 63.5	54.2	3.31
Chest Circum (cm)	56.0 – 64.5	60.0	2.61
Belly Circum (cm)	55.0 – 64.0	59.7	3.25
Ave Back Fat (mm)	5.7 – 7.5	6.3	0.61

All values were obtained from 12 pigs

TABLE 8. Red Cell, Plasma and Blood Volume Characteristics of Immature Domestic Swine

Pig	RCV (ml/kg)	PV (ml/kg)	BV (ml/kg)	Body Hct	Art Hct	F_{cells}
15	19.6	47.0	66.6	0.29	0.35	0.83
18	16.7	72.6	89.3	0.19	0.29	0.66
19	17.6	71.0	89.5	0.20	0.30	0.69
20	15.7	66.1	78.5	0.20	0.30	0.66
21	17.7	72.7	86.7	0.20	0.31	0.64
22	15.0	64.7	79.7	0.19	0.27	0.70
24	16.4	59.6	76.0	0.22	0.30	0.72
25	19.1	73.0	92.1	0.21	0.30	0.70
mean	17.2	66.5	82.3	0.21	0.31	0.70
S.D.	1.59	8.66	8.65	0.03	2.52	0.06

Abbreviations: RCV, red cell volume; PV, plasma volume; BV, blood volume; and F_{cells}, ratio of Body Hct to Arterial Hct.

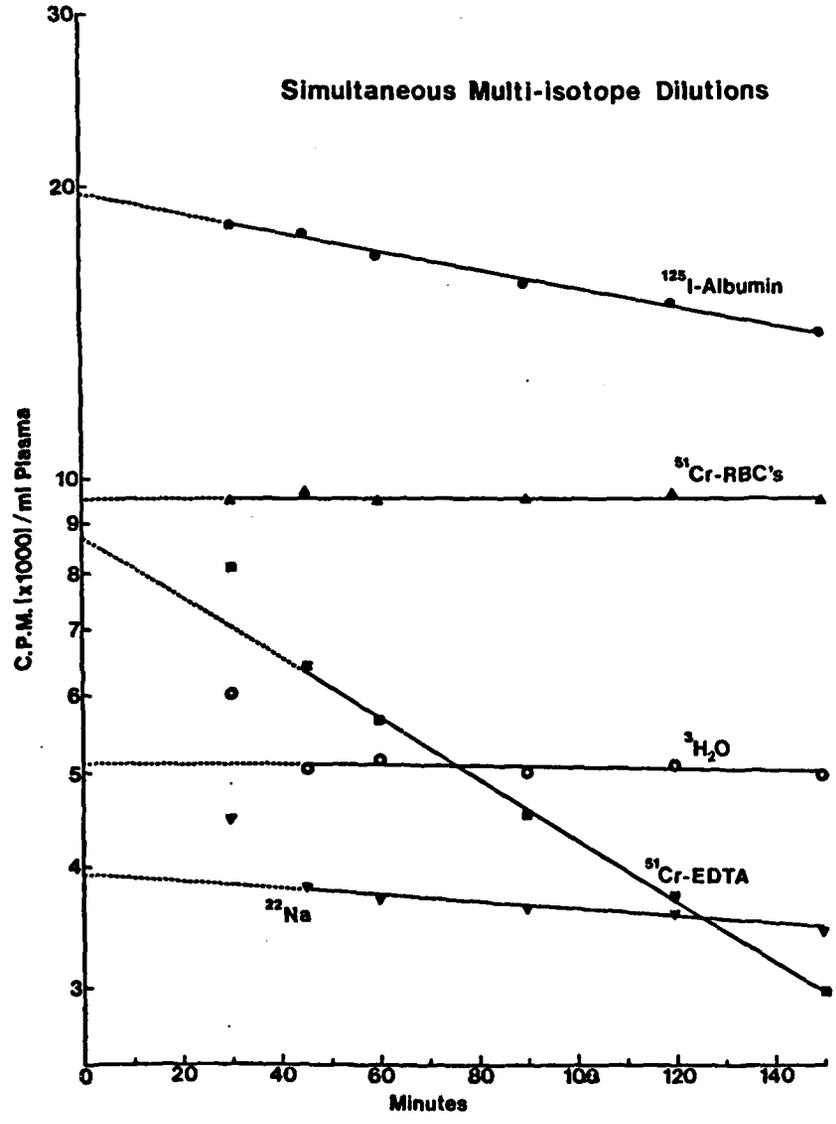


Figure 11. Simultaneous dilution measurements of 5 radiolabeled indicators in the arterial plasma of a conscious pig. Red cell volume is estimated with ^{51}Cr-RBC's, plasma volume with ^{125}I-albumin, extracellular space with ^{51}Cr-EDTA and ^{22}Na, and total body water with $^{3}H_2O$.

REFERENCES

1. Moulton CR. Age and chemical development in mammals. J Biol Chem 1923;57:79-97.

2. Blaxter KL, Rook JAF. The indirect determination of energy retention in farm animals: II. Studies on the validity of the method J Agric Sci 1956;48:210-217.

3. Etherton TD, Wangness PJ, Hammers VM, Ziegler JH. Effect of dietary restriction on carcass composition and adipocyte cellularity of swine with different propensities for obesity. J Nutr 1982;112:2314-2323.

4. Pace N, Rathbun E. Studies on body composition: III. Body water and chemically combined nitrogen content in relation to fat. J Biol Chem 1945;158:685-691.

5. Kleiber M. The fire of life: an introduction to animal energetics. New York, NY: John Wiley and Sons, Inc, 1961.

6. Behnke A, Osserman EF, Welham WC. Lean body mass. Arch Intern Med 1953;91:585-601.

7. Moore FD, Boyden CM. Body cell mass and limits of hydration of the fat-free body. Their relation to estimated skeletal weight. Ann NY Acad Sci 1963;110:62-71.

8. Shires T, Williams J, Brown J. Simultaneous measurement of plasma volume extracellular fluid volume and red blood cell mass in man utilizing I^{131}, $S^{35}O_4$, and Cr^{51}. J Lab Clin Med 1960;55:776-783.

9. Fogelman MJ. Internal water exchange rates following hemorrhage in splenectomized dogs. Am J Physiol 1952;169:94-101.

10. Association of Official Agricultural Chemists. Official methods of analysis. 10th ed. Washington, DC; 1965.

11. Doornenbal H. Growth development and chemical composition of the pig. III. Bone ash and moisture. Growth 1975;39:427-434.

12. Brooks CC, Fontenot JP, Vipperman PE, Thomas HR, Graham PP. Chemical composition of the young pig carcass. J Anim Sci 1964;23:1022-1026.

13. Shields RG, Mahan DC, Graham PP. Changes in swine body composition from birth to 145 kg. J Anim Sci 1983;57:43-54.

14. Mersmann HJ. Ultrasonic determination of back fat depth and loin area in swine. J Anim Sci 1982;54:268-275.

15. Jones SDM, Haworth CR. Instrument prediction of the lean content of pork carcasses using ultrasound or light reflectance. J Anim Sci 1983;57:418-425.

16. Domermuth W, Veum TL, Alexander MA, Hedrick HB, Clark J, Eklund. Prediction of lean body composition of live market weight swine by indirect methods. J Anim Sci 1976;43:966-976.

17. Ward GM, Johnson JE. Nuclear techniques for in vivo determination of body compostion: A review. In: Isotope studies on the physiology of domestic animals. Proceedings of the symposium on use of isotopes in studies on physiology of domestic animals; 1972 March 20-24; Athens. Vienna:International Atomic Energy Association, 1972:3-20.

18. Harrison GG, Van Itallie TB. Estimation of body composition: a new approach based on electromagnetic principles. Am J Clin Nutr 1982;35:1176-1179.

19. Lawson H. The volume of blood-a critical examination of methods for its measurement. In: Handbook of Physiology Section 2 Circulation Washington, DC: American Physiology Society, 1962:23-49.

20. Kay M, Jones AS, Smart R. The use of tritiated water 4-amino antipyrene and N-acetyl-4-amino antipyrene for the measurement of body water in living pigs. Br J Nutr 1966;20:439-448.

21. Sheng HP, Huggins RA. A Review of body composition studies with emphasis on total body water and fat. Am J Clin Nutr 1979;32:630-647.

22. Sterling K, Gray SJ. Determination of the circulating red cell volume in man by radioactive chromium. J Clin Invest 1950;29:1614-1619.

23. Nelson AW, Swan H. Blood volume II. Precise long term measurements using chromium-tagged erythrocytes. Ann Surg 1971;173:496-503.

24. Swan H, Nelson AW. Blood volume I: Critique: Spun vs Isotope hematocrit; ^{125}RIHSA vs ^{51}CrRBC. Ann Surg 1971;173:481-495.

25. Burch GE, Threefoot SA, Ray CT. Rates of turnover and biological decay of chloride and chloride space in the dog determined with the long life isotope ^{36}Cl. J Lab Clin Med 1950;35:331-347.

26. Walser M. Volume of distribution of radiosulfate as a measure of the extracellular fluid. Proc Soc Exp Biol Med 1952;79:372-375.

27. McMurrey JD, Boling EA, Davis JM, Parker HV, Magnus IC, Ball MR, Moore FD. Body composition: Simultaneous determination of several aspects by the dilution principle. Metabolism 1958;7:651-667.

28. Addanke S, Cahill FD, Sotos JF. A method for the determination of extracellular space with ^{3}H-inulin. Clin Chem 1967;13:953-957.

29. Virgilio RW, Homer LD, Herman CM, Moss GS, Lowery BD, Schwartz SL. Comparison of inulin and chromium-EDTA spaces in the nephrectomized baboon. J Surg Res 1970;10:370-375.

30. Hansard SL, Sauberlich HE, Comar CL. Blood volume of swine. Proc Soc Exp Biol Med 1951;78:544-545.

31. Talbot RE, Swenson MJ. Measurement of porcine plasma volume using T-1824 dye. Am J Vet Res 1963;24:467-471.

32. Deavers SI, Huggins RA, Sheng HP. Blood volume changes during the first week after birth in the beagle and pig. Proc Soc Exp Biol Med 1978;159:152-156.

33. Cummings JN. Fetal anemia and hypovolemea in swine. Can Vet J 1968;9:61-69.

34. Doornenbal H, Asdell SA, Wellington GH. Chromium-51 determined red cell volume as an index of "lean body mass" in pigs. J Anim Sci 1962;21:461-463.

35. Talbot RB, Swenson MJ. Blood volume of pigs from birth through 6 weeks of age. Am J Physiol 1970;218:1141-1144.

36. Bush JA, Jensen GE, Cartwright GE, Wintrobe MM. Blood volume studies in normal and anemic swine. Am J Physiol 1963;181:9-14.

37. The Health Physic and Radiological Health Handbook. Shlein B, Terpilak MS. eds Rockville: Lucleon Lectern Assoc. Inc. 1984.

38. Setiabudi M, Sheng HP, Huggins RA. Growth of the pig: changes in red cell and plasma volumes. Growth 1976;40:127-132.

39. Hannon JP, Bossone CA, Rodkey WG. Splenic red cell sequestration and blood volume measurements in conscious pigs. Am J Physiol 1985;248:R293-R301.

40. Kraybill HF, Goode ER, Robertson RSB, Sloane HS. In vivo measurement of body fat and body water in swine. J Appl Physiol 1954;6:27-32.

41. Hansard SL. Total body water in farm animals. Am J Physiol 1964;206:1369.

42. Wood AJ, Groves TDD. Changes in body composition of the pig during early growth based on deuterium oxide dilution technique. Ann NY Acad Sci 1963;110:349-357.

43. Flynn MA, Hanna F, Long CH, Asfour RY, Lutz RN, Zorbrisky SE. Deuterium oxide as a predictor of body composition in children and pigs. In: Brozek J. ed. Body composition in animals and man. National Acad Sci publ. 1598 1968:480-491.

44. Sheng HP, Kanonsakpithak MS, Naiborhu A, Chuntananukoon S, Huggins RA. Measured and calculated fat during growth in the pig and beagle. Growth 1977;41:139-146.

45. Setiabudi MS, Kamonsakpithak MS, Sheng HP, Huggins RA. Growth of the pig: Changes in body weight and body fluid compartments. Growth 1975;39:405-415.

46. Pond WG, Houpt KA. Biology of the Pig. Ithaca, NY: Cornell Univ Press, 1978.

47. Hornicke VH. Methoden zur bestimmung der körperzusammensetzung lebender tiere unter besonderer Berücksichtigung des Schweines. Z Tierphys Futter 1962;17:28-61.

48. Remy J, Daburon F, Nizza P. Les compartments corporels chez le porc. In: Isotope Studies on the Physiology of Domestic Animals Proceedings of the symposium on use of isotopes in studies on the physiology of domestic animals, 1972 March 20-24, Athens. Vienna: International Atomic Energy Association, 1972:35-47.

49. Moore FD, McMurray JD, Parker HV, Magnus IC. Body composition, total body water, and electrolytes: Intravascular and extravascular phase volumes. Metabolism 1956;5:447-467.

50. Burke BJ, Staddon GE. The precision of a modern method for body compartment measurements using multiple isotopes. Clinica Chimica Acta 1981;117:85-95.

51. Baker CH. ^{51}Cr-labeled red cell, ^{131}I-fibrinogen and T-1824 dilution spaces. Am J Physiol 1963;204:176-180.

52. Wood AJ, Grooves TDD. Body composition studies on the suckling pig. I. Moisture, chemical fat, total protein, and total ash in relation to age and body weight. Can J Anim Sci 1965;45:8-13.

53. Maksoud JG, Kieffer J. Kinetics of the extracellular space in newborn animals submitted to surgical trauma. J Surg Res 1975;19:259-266.

54. Pierson RN, Price DC, Wang J, Jain RK. Extracellular water measurements organ tracer kinetics of bromide and sucrose in rats and man. Am J Phys 1978;235:254-264.

55. Figueras HD, Verges JB, Carou N, Parodi JJ, Garcio Olanw H, Marangunich L, Cavandoli H, Marcilese NA. Evaluation of radioisotope dilution to estimate the body composition of sheep in vivo. In: Isotope Studies on the Physiology of Domestic Animals. Proceedings of the Symposium on use of Isotopes in studies on Physiology of Domestic Animals, 1972 March 20-24, Athens. Vienna, International Atomic Energy Association, 1972:49-62.

56. Levitt MF, Gaudino M. Use of radioactive isotopes to measure intracellular cation concentration in the normal dog. Am J Phys 1949;159:67-72.

57. Balkissoon B, Spellman MW, Hawthorne EW. Blood volume intracellular and extracellular fluid spaces in normal dogs and in dogs with ascites secondary to severe tricupsid insufficiency. Am J Phys 1956;184:282-286.

58. Harrison CS, Becker JM. Determination of deuterium oxide and sucrose spaces in untreated burned dogs. Surgery 1957;41:636-644.

59. Pierson RN, Wang J, Colt EW, Neumann P. Body composition measurements in normal man: The potassium sodium sulfate and tritium spaces in 58 adults. J Chron Dis 1982;35:419-428.

60. Steinhardt M, Steinhardt K, Wachtel W, Lyhs L. Untersuchungen über das plasmavolumen blutvolumen und die totale hämoglobinmenge bei schweinen. Archiv Exper Vetmed 1972;26:533-550.

61. Linderkamp O, Berg D, Betke K, Koferl F, Friegel H, Riegel KP. Blood volume and hematocrit in various organs in newborn piglets. Pediat Res 1980;14:1324-1327.

62. Clawson AJ, Sheffy BE, Reid JT. Some effects of feeding chlortetracycline upon the carcass characteristics and the body composition of swine and scheme for the resolution of the body composition. J Anim Sci 1955;14:1122-1132.

63. Nelson AW, Swan H. The effects of the canine spleen on red cell and blood volume determinations using ^{51}RBC[sic]. Surgery 1972;71:215-224.

64. Breznock EM, Strack D. Effects of the spleen, epinephrine and splenectomy on determination of blood volume in cats. Am J Vet Res 1982;43:2062-2066.

65. Reeve EB, Gregersen MI, Allen TH, Sear H. Distribution of cells and plasma in the normal and splenectomized dog and its influence on blood volume estimates with P^{32} and T1824. Am J Phys 1953; 175:195-203.

66. Chaplin H, Mollison PL, Vetter H. The body/venous hemotocrit ratio: its constancy over a wide hematocrit range. J Clin Invest 1953;32:1309-1316.

67. Lipowski HH, Usami S, Chien S. In vivo measurements of "apparent viscosity" and microvessel hematocrit in the mesentery of the cat. Microvasc Res 1980;19:297-319.

68. Elkinton JR, Danowski TS. The Body Fluids. Basic physiology and practical therapeutics. Baltimore, MD: The Williams and Wilkins Co, 1955.

69. Winkler AW, Elkinton JR, Eisenman AJ. Comparison of sulfocyanate with radioactive chloride and sodium in measurement of extracellular fluid. Am J Physiol 1943;139:239-246.

70. Swan H, Nelson AW, Hankes GH. Radio-chloride (^{36}Cl) as a continuous monitor of extracellular water in the dog. Ann Surg 1971;174:287-295.

71. Gamble JL, Robertson JS, Hannigan CA, Foster CG, Farr LE. Chloride, bromide, sodium and sucrose spaces in man. J Clin Inv 1953;32:483-489.

72. Ladegaard-Pedersen HJ, Engell HCA. A comparison of the distribution volumes of inulin and [^{51}Cr] EDTA in man and nephrectomized dogs. Scand J Clin Lab Invest 1972;30:267-270.

73. Darrow DC, Hellerstein S. Interpretation of certain changes in body water and electrolytes. Physiol Rev 1958;38:114-137.

74. Kulwich R, Struglia L, Pearson PB. Metabolic fate of S^{35}-labeled sulfate in baby pigs. Proc Soc Exp Biol Med 1958;97:408-410.

75. Coleman TG, Manning RD, Norman RA, Guyton AC. Dynamics of water-isotope distribution. Am J Physiol 1972;223:1371-1375.

76. Moore FD. Determination of total body water and solids with isotopes. Science 1946;104:157-161.

77. Prentice TC, Siri W, Berlin NI, Hyde GM, Parsons RJ, Joiner EE, Lawrence JH. Studies of total body water with tritium J Clin Invest 1952;31:412-418.

78. Hansard SL. Radiochemical procedures for estimating body composition in animals. Ann NY Acad Sci 1963;110:229-245.

79. Pace N, Kline L, Schachman HK, Harfenist M. Studies on body composition IV. Use of radioactive hydrogen for measurement in vivo of total body water. J Biol Chem 1947;168:459-469.

80. Sheng HP, Huggins RA. Direct and indirect measurement of total body water in the growing beagle. Proc Soc Exptl Biol and Med 1971;137:1093-1099.

81. Nagy KA, Costa DP. Water flux in animals: analysis of potential errors in the tritiated water method. Am J Physiol 1980;238:R454-R465.

82. Tisavipat A, Vibulsreth S, Sheng HP, Huggins RA. Total body water measured by desiccation and by tritiated water in adult rats. J Appl Physiol 1974;37:699-701.

83. Vaughan BE, Boling EA. Rapid assay procedures for tritium-labeled water in body fluids. J Lab and Clin Med 1961;57:159-164.

84. Doornenbal H, Martin AH. The evaluation of blood volume and total red cell mass as predictors of gross body composition in the pig. Can J Anim Sci 1965;45:203-210.

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